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In re Applicant:

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ZAMIR et al §
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§ Dorakin
For: Polynucleotide Encoding Polypeptides § Attorney
Having Invertase Activity and § Docket: **02/23531**
Use of Same §
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Examiner: Leonard E. Smith §

Director of the United States Patent and Trademark Office
Washington, D.C. 20231

## TRANSMITTAL OF PRIORITY DOCUMENT

Sir:

Further to the Decision on Petition letter dated August 13, 2002, we now

enclose a certified copy of U.S. Patent Application 09/477,375 in support of the

perfection of the priority date claim.

Respectfully submitted,

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Date: October 28, 2002

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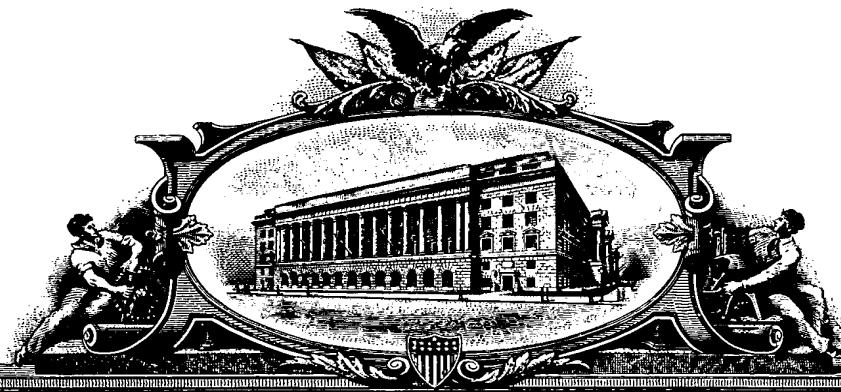
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September 06, 2002

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APPLICATION NUMBER: *09/477,375*
FILING DATE: *January 04, 2000*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

T. LAWRENCE
Certifying Officer

5

10   Inventors: Dani Zamir, Tzili Pleban and Eyal Fridman

15

POLYNUCLEOTIDES ENCODING POLYPEPTIDES
HAVING INVERTASE ACTIVITY AND USE OF SAME

20

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to isolated polynucleotides encoding

25   polypeptides having invertase activity, constructs including same and

methods of utilizing same.   More particularly, the present invention

relates to isolated polynucleotides encoding novel invertases, which

polynucleotides can be used for substantially increasing the sugar content

in for example, fruits, roots, leaves, etc., of plants expressing same.   In

30   addition, the present invention relates to a novel regulatory sequence

which when integrated, in a site specific manner, into a *solanaceae* plant

THE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

Case Docket No.: 325/78

Transmitted herewith for filing is the patent application of

Inventor: DANI ZAMIR ET AL.

For: POLYNUCLEOTIDES ENCODING POLYPEPTIDES HAVING INVERTASE ACTIVITY AND USE OF SAME

Enclosed are:

☒ ___ sheets of _informal_ drawing(s).

☒ An assignment of the invention to YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM

☐ A certified copy of a _____ application.

☐ An associate power of attorney.

☒ A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.

☒ Other - SEQUENCE LISTING IN A COMPUTER READABLE FORM

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TOTAL CLAIMS	38 - 20=	18	18 x9=	$ 162	OR	x18=	$
INDEP CLAIMS	15 - 3=	12	12 x39	$ 468	OR	x78	$
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☒ Any filing fees under 37 CFR 1.16 for presentation of extra claims.

Respectfully,

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genome, can substantially increase the sugar content in tissues, such as for example, fruits, etc., of the solanaceae plant.

Ever since the emergence of modern agriculture, agricultured plants have been manipulated in an effort to establish crops with agronomically important traits.

Such traits typically include, plant yield and quality, enhanced growth rates and adaptation to various growth conditions.

At present, a great deal of emphasis is placed on the generation of plants having desired traits via genetic engineering techniques. However, since these traits are often the result of the activity of several genes (referred to as quantitative traits), the use of direct gene transfer in manipulating these traits, is difficult due to problems in pinpointing and then cloning the individual loci which contribute predominantly to the expression of the trait.

As such, genetic manipulation of plants is typically practiced using conventional breeding techniques, such as hybrid crossing.

Although utilizing such breeding techniques typically enables breeders to interogress quantitative trait loci of a specific function into a

desirable genetic background, such conventional breeding techniques suffer from several limitations.

Oftentimes the "isolation" of a single trait loci (referred to as quantitative trait loci or QTL) can be difficult due to linkage drag, or due to effects of epistatic QTLs present in the genetic background or in chromosomal association with the single trait loci.

One example of a plant which has been extensively bred is tomato.

A major objective in tomato breeding is to increase the content of total soluble solids (TSS or brix; mainly sugars and acids) of the fruits in order to improve taste and processing qualities.

As such, efforts have been made to interogress the high fruit sugar content of wild *Lycopersicon* species which is three times higher than cultivated varieties into cultivated varieties which are characterized by a large fruit mass, small foliage, concentrated ripening and other commercially desirable traits.

To try and resolve the genetic basis for the high sugar content of fruits of wild *Lycopersicon* species, Eshed and Zamir (Genetics 141: 1147-1162, 1995; Genetics 143: 1807-1817, 1996, both are incorporated herein by reference) developed a set of 50 introgression lines from a

cross between the green-fruited species *L. pennellii* and the cultivated tomato, *L. esculentum.* Each of the lines contained a single RFLP defined *L. pennellii* chromosome segment, and together the lines provide complete coverage of the tomato genome. Using this resource it was

5   possible to map 23 QTLs that regulate brix.

Although this research work presents significant progress in determining the QTLs responsible for a high brix value, plants generated by introgressing *L. pennellii* into the cultivated tomato, *L. esculentum* genetic background are of little commercial value since their phenotype,

10   in many aspects, is closer to that of the wild *Lycopersicon* species.

In order to generate hybrids characterized by a uniform ripening, a good cover of the fruit and a high brix value, which hybrids are of high commercial value, it is necessary to narrow the introgression described by Eshed and Zamir in order to isolate the brix QTL.

15   Furthermore, since genetic crossing is genus limited, in order to enable generation of a high brix value in plants unbreedable with tomato plants, a gene or genes responsible for the high brix value in *L. pennellii* must be isolated, which gene or genes when introduced and expressed in

a plant other than tomato substantially increase the fruit brix value thereof.

Thus, the present invention describes the isolation of polynucleotides which encode for novel plant invertases which are associated with the high brix value in *L. pennellii* fruit. The present invention further describes recombinant methods which utilize these isolated polynucleotides for increasing the brix value of plant tissues.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided an isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino acid sequence serving for secretion into an apoplast.

According to further features in preferred embodiments of the invention described below, the polypeptide is at least 80 % homologous to SEQ ID NOs:6 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman

algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

According to still further features in the described preferred embodiments the polynucleotide is at least 80 % identical with SEQ ID NOs:7 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to another aspect of the present invention there is provided an isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity, the polypeptide is at least 80 % homologous to SEQ ID NOs:6, 12 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

According to further features in preferred embodiments of the invention described below, the polynucleotide is hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 under hybridization conditions of hybridization

solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

According to still further features in the described preferred embodiments the polynucleotide is at least 80 % identical with SEQ ID NOs:7, 9 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to still further features in the described preferred embodiments the polypeptide is as set forth in SEQ ID NOs:6, 12 or 13 or portions thereof.

According to still further features in the described preferred embodiments the polynucleotide is as set forth in SEQ ID NOs:7, 9 or 11 or portions thereof.

According to yet another aspect of the present invention there is provided a nucleic acid construct comprising any of the isolated nucleic acids described hereinabove.

According to still further features in the described preferred embodiments the nucleic acid construct further comprising a promoter for regulating expression of the isolated nucleic acid in an orientation selected from the group consisting of sense and antisense orientation.

5   According to still further features in the described preferred embodiments the nucleic acid construct further comprising a positive and a negative selection markers for selecting for homologous recombination events.

According to still another aspect of the present invention there is 10 provided a plant cell, tissue or a whole plant comprising any of the nucleic acid constructs described herein.

According to an additional aspect of the present invention there is provided a recombinant protein comprising a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino 15 acid sequence serving for secretion into an apoplast.

According to still further features in the described preferred embodiments the polypeptide is at least 80 % homologous to SEQ ID NOs:6 or 13, as determined using the BestFit software of the Wisconsin

sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

According to still further features in the described preferred embodiments the polypeptide includes at least a portion of SEQ ID NOs:6 or 13.

According to still further features in the described preferred embodiments the protein is encoded by a polynucleotide hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 or a portion thereof under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

According to still further features in the described preferred embodiments the protein is encoded by a polynucleotide at least 80 % identical with SEQ ID NOs:7 or 11 or portions thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to still further features in the described preferred embodiments the recombinant protein comprising a polypeptide as set forth in SEQ ID NOs:6, 12 or 13.

According to yet an additional aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing in the plant tissue a polypeptide having invertase activity, wherein the polypeptide is at least 80 % homologous to SEQ ID NOs:6, 12 or 13 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

According to still an additional aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing a polypeptide having invertase activity, wherein the polypeptide is encoded by a polynucleotide hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 or a portion thereof under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm

^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

According to another aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing a polypeptide having invertase activity, wherein the polypeptide is encoded by a polynucleotide at least 80 % identical with SEQ ID NOs:7, 9 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to yet another aspect of the present invention there is provided an isolated regulatory element comprising a polynucleotide at least 50 % identical with SEQ ID NO:4 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to still another aspect of the present invention there is provided an isolated regulatory element comprising a polynucleotide

hybridizable with SEQ ID NO:4 under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 1 x SSC and 0.1 % SDS and final wash at 50 °C.

5   According to an additional aspect of the present invention there is provided an expression vector including the isolated regulatory element described herein.

According to yet an additional aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a

10   tissue of a solanaceae plant, the method comprising the step of integrating into a genome of the solanaceae plant a polynucleotide including a nucleic acid sequence as set forth in SEQ ID NO:4, wherein said polynucleotide is integrated into a specific site of chromosome 9 of the solanaceae plant via homologous recombination.

15   According to still an additional aspect of the present invention there is provided a method for determining whether fruits to be produced from solanaceae seeds or solanaceae seedling will contain an amount of monosaccharides above a predetermined threshold, the method comprising the step of detecting the presence or absence of a nucleic acid

sequence as set forth in SEQ ID NO:4 in genomic DNA derived from the solanaceae seeds or solanaceae seedling.

The present invention successfully addresses the shortcomings of the presently known configurations by providing means with which the monosaccharide content of plant tissue or organ can be increased.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings

making apparent to those skilled in the art how the several forms of the

invention may be embodied in practice.

In the drawings:

FIGs. 1a-i depict the chromosomal locations, sizes and identities

5   of the 50 *L. pennellii* introgression lines (ILs) on chromosomes 1-12.

The genetic map was constructed on the basis of 119 BC1 plants as

described by Eshed and Zamir (1995). Mapped markers which are

associated with the chromosome of a plant line, and markers not assayed

on the BC1 map are placed according to their approximate positions

10   based on Tanksley et al. Each line was probed with all the markers, and

lines showing wild-species alleles are marked with bars to the left of the

chromosome. e - *L. esculentum*, p - *L. pennellii* (Prior art).

FIG. 2 depicts the digenic interactions between unlinked QTLs.

The values on the left and at the top of the Figures are the difference (in

15   %) of each IL hybrid (ILH) from M82 according to Table 2 below.

Values in bold are significant at $p < 0.05$ (Dunnet's t test). Each histogram

represents the difference (in %) of the hybrid heterozygous for the two

introgression from the sum of the effect of the two individual ILHs for all

traits measured (PW- plant weight, FM- fruit mass, B- brix, Y- total fruit

yield, BY- the product of B and Y). Bars in white show no significant interaction and bars in light gray, gray and black indicate significant interactions of $p<0.05$, $p<0.01$ and $p<0.001$, respectively (prior art).

FIG. 3 depicts the distribution of the observed and expected numbers of pairs of introgressions showing simultaneous significant epistasis ($p<0.05$) for the traits: plant weight (PW), fruit mass (FM), brix (B) and yield (Y). The expected values were calculated on the basis of complete independence between traits and a mean epistatic rate of 0.28 for each trait (prior art).

FIG. 4 depicts the relationship between the expected an observed values for plant weight, fruit mass, brix, yield, and brix x yield of 45 hybrids of two ILs. Expected values were calculated on the basis of complete additivity of the effects of the individual ILHs. (prior art).

FIG. 5 depicts the fine mapping of linked QTLs for B and FM on the long arm of chromosome 2. The dark bars represent the *L. pennellii* chromosome segments introgressed into M82. Each point is the mean of the estimated introgression effect; bars represent the standard errors of the means. The mean phenotypic value of each line was determined as described in example 2 of the Examples section that follows (prior art).

FIG. 6 is a photograph depicting the fruit size of lines used for the mapping analysis of the linked QTL on chromosome 2. Top, IL2-5. Second row: left, IL2-5-1; right, IL2-6-1. Third row: left, IL2-5-3; center, IL2-6-6; right, IL2-6-4. Bottom, M82 (prior art).

5   FIG. 7 depicts an interaction between IL9-2-5 and the year grown as expressed by plant weight (PW), fruit mass (FM) and brix (B). The values of IL9-2-5 and the hybrid ILH9-2-5 over the years 1995, 1996 and 1997 are expressed as the percent difference from the isogenic control M82 (Δ% of M82). Results for IL9-2-5 are indicated by the gray bars

10   while results for ILH9-2-5 are indicated by the ladder bars. * above the bars denotes a significance difference (p<0.01) from the control and * in the d values represents a significant (p<0.05) dominance deviation of the heterozygous. For the traits showing no yearly dependence (alpha level = 0.01) data from the three years was pooled to estimate a and d. The mean

15   and the standard deviation values for M82 are indicated at the bottom of the Figure; PW- Kg, FM-g; B-%.

FIG. 8a depicts a genetic map of the IL9-2-5 introgression. The genetic distance in centimorgans (cM) is indicated between each pair of markers and is based on the F2 population. The genotype of IL9-2-5,

IL9-2-6 and IL9-2-7 is represented by a hatched bar (*L. pennellii*) and an empty bar (*L. esculentum*). The border between the two bars is determined arbitrary between the two flanking markers.

FIG. 8b depicts the phenotypic effects of the IL9-2-5 (ladder bar), IL9-2-6 (black) and IL9-2-7 (white) hybrids compared to the control M82. * and [+] above the bars denote a significance difference ($p<0.05$, $p<0.1$, respectively) from the control. Each value represents the mean of eight plots.

FIG. 9 is a scatter plot depicting brix values of two isogenic hybrids, M82 × line 202 (17 plants) and IL9-2-5 × line 202 (8 plants). The center lines of the means (diamonds) are the group means. The top and bottom of the diamonds form the 95 % confidence intervals for the means. sp9 - a novel tomato marker (SEQ ID NO:16 as a probe and *Eco*RV as a restriction enzyme).

FIG. 10 is a schematic depiction of the IL9-2-5 and IL-9-2-4 introgressions with respect to tomato chromosome 9.

FIG. 11 is a collection of scatter plots depicting the effects of brix 9-2-5 in the 3-year trial of the indeterminate (glasshouse) NILs. e - *L. esculentum*, p - *L. pennellii*. The homozygous IL (*pp*), containing

segment of chromosome 9, improved B by 27 percent over the control

(*ee*) with partial dominance for the wild species segment (*ep*) (a=0.5,

d=0.25, d/a=0.5). Black arrows and horizontal gray lines mark the mean

values and the 99.9 % confidence interval for each genotype.

5   FIG. 12 is a collection of scatter plots depicting the effects of

Brix9-2-5 in the 3-year trial of the determinate NILs. *ee, pp* and *ep*

represent NILs homozygous for the *L. esculentum* allele *Brix9-2-5*, NILs

homozygous for the *L. pennellii* allele, and heterozygous NILs,

respectively. Black arrows and horizontal gray lines mark the mean

10   values and the 99.9 % confidence interval for each genotype.

FIG. 13 depicts the fine-mapping and physical positioning of

Brix9-2-5 on chromosome 9. The upper portion shows the genetic

linkage map (in cM) of the chromosomal region of *Brix9-2-5*, wherein

the two end clones of BAC91A4, 91N and 91S, are indicated in boxes.

15   The mid portion shows the genetically ordered markers on BAC91A4

and the number of recombinants between them. The lower portion shows

the recombination groups in the BAC. Each group in the lower portion is

composed of families with a common introgressed segment and is

represented by a divided bar of hatched (*L. pennellii*) and empty (*L.*

esculentum) genomic segments. The borders between bars are arbitrarily drawn midway between markers positive and negative for the introgressed *L. pennellii* segment.

FIG. 14a depicts the nucleotide polymorphism (NP) and phenotypic analysis of 13 recombinant families of *Brix9-2-5*. Each NP is represented by a nucleotide number and the corresponding *L. pennellii* (top) and *L. esculentum* nucleotides. Full black bars denote a significant phenotypic effect (p<0.001). * denotes a verification of recombinant family 6 (- 3 %, nor significant) in an F4 generation.

FIG. 14b depicts the genomic structure of the *Lin5* gene. Boxes depict exons and the arrows represent the recombination points for each of the individual recombinant families (numbered as in Figure 14a), the nucleotide sequence of the *L. pennellii Lin5* region spanning the QTL is presented by nucleotides 2301-2850, which are numbered to correspond to the start codon of *Lin5*. NPs between the two species are shown in bold and the codons for the 3 amino acids substitutions are underlined: positions 2403 (Asp in *L. pennellii* to Glu in *L. esculentum*), 2457 (Asp to Asn) and 2478 (Val to Leu); the intron sequence is depicted by outlined letters. Deleted nucleotides in the *L. esculentum* sequence are

boxed and a four bp insertion (ATCT) following base 2735 is indicated by a [V]. The 18-bp direct repeat is double underlined and the 7-bp repeats are marked with a wavy line. The start and stop codons of a hypothetical intraintronal open reading frame are denoted by dashed boxes.

5   FIG. 15 is a schematic depiction of the Lin5 and Lin7 exons intron structure.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

10   The present invention is of isolated polynucleotides encoding novel plant invertases which can be used to increase the monosaccharide content in plants transformed therewith. Specifically, the present invention can be used to increase the monosaccharide content in plant tissues, such as, for example, fruits, leaves or roots by expressing at least

15   one of the isolated polynucleotides which encode said novel plant invertases within the plant. The present invention is further of a novel plant expression regulatory element which can be used to increase the monosaccharide content in fruits of plants into which this regulatory

element is genomically integrated in a site specific manner, especially in solanaceae.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Edible plant tissues, such as fruit, which store a high level of monosaccharides are a particularly sought after for both commercial processing and personal consumption.

As such, plant breeding techniques are often used by plant breeders in order to transfer such a desirable trait into cultivated species.

However, plant breeding techniques can only be used for related plant species and as such this desired trait cannot be transferred between unrelated plant species.

As such, the isolation of a gene or genes which are responsible for this trait is necessary such that recombinant techniques can be used to introduce this trait into a wide range of plants.

One family of genes which are responsible for monosaccharide generation in plants are the extracellular invertases.

Extracellular invertases enzymes are hydrolases, cleaving sucrose to glucose and fructose, which are transported into the cells. This activity maintains a gradient of assimilates, from the source parts of the plant, to the developing sink tissues. Cell wall invertases are synthesized as preproteins, with a long leader sequence which is cleaved off during transport and protein maturation. All known cDNA-derived amino acid sequences of invertases possess a signal peptide, required for entry into the endoplasmatic reticulum (ER) and, thus, into the secretory pathway. The mature peptide includes the NDPNG (SEQ ID NO:14) and WECPDF (SEQ ID NO:15) sequences which form the β-fructosidase motif and the catalytic site, respectively.

In tomato, the apoplastic invertase isoenzymes are encoded by a gene family comprising four members: *Lin 5*, *Lin 6*, *Lin 7* and *Lin 8* (Godt and Roitch, Plant Physiol. 115, 273-282, 1997). The published sequences of this gene family are mostly of the third and biggest exon of

5 the gene, exon 3, and the full sequence of each of these genes remains undetermined.

As further detailed hereinbelow in Examples 3-5 of the examples section, while reducing the present invention to practice, a carefully planned approach using marker selected breeding of tomato plant

10 introgression lines (ILs) enabled the determination of a narrow chromosomal region which is associated with the high level of monosaccharide accumulation (brix value) in *L. pennellii* fruits. Sequencing of a bacterial artificial chromosome (BAC) which includes this region has revealed the existence of two novel invertase genes which

15 display identity to previously published *Lin5* and *Lin 7* partial cDNA sequences and which are termed herein as pLin5 and eLin7, respectively.

Comparison to *L. esculentum* sequences of the same chromosomal region has also revealed the existence of a sequence unique to the *L. pennelli* chromosomal sequence. This sequence which is 484 base pairs

long spans a portion of the genomic polynucleotide sequence of pLin5 which includes a 3' portion of exons 3, intron 3 and a 5' portion of exon 4 of pLin5.

As further detailed in Example 5 of the Examples section, this sequence functions in either regulating the expression of pLin5 and/or eLin7 or in directing the co-splicing of exons 1 and 2 from pLin5 with exons 3-6 from eLin7 to form a chimeric invertase transcript.

As further detailed in these examples, the various isoenzymes which are optionally produced from the transcripts of pLin5 and eLin7 and/or from the chimeric transcript function either independently or cooperatively in contributing to the high brix value associated with *L. pennellii* fruits.

Thus, according to one aspect of the present invention there is provided an isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino acid sequence serving for secretion into an apoplast.

As used herein in the specification and in the claims section that follows, the phrase "complementary polynucleotide sequence" includes

sequences which originally result from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. Such sequences can be subsequently amplified *in vivo* or *in vitro* using a DNA dependent DNA polymerase.

As used herein in the specification and in the claims section that follows, the phrase "genomic polynucleotide sequence" includes sequences which originally derive from a chromosome and reflect a contiguous portion of a chromosome.

As used herein in the specification and in the claims section that follows, the phrase "composite polynucleotide sequence" includes sequences which are at least partially complementary and at least partially genomic.

The phrase "having an invertase activity in an apoplastic environment" is used herein to distinct cellular invertases from those secreted into the apoplast. Plant invertases are characterized by their subcellular localization, their pH optima and their characterizing isoelectric point, pI. Intracellular invertase are characterized by acidic pH optima and low pI and are thought to be in the vacuole, whereas extracellular invertases are also characterized by acidic optima but a high

pI that enables its bounding to the negatively charged cell-wall. Comparison of the known plant invertase genes revealed at least two distinguishing motifs: (i) Cell-wall invertases carry the amino acid Proline in their β-fructosidase motif (WEC<u>P</u>DF, SEQ ID NO:15), as is compared to Valine in the vacuolar peptide; and (ii) in contrast to the cell wall invertases, the intracellular invertases contain an additional C-terminal extension, which might be involved in the vacuolar targeting of the protein.

According to one preferred embodiment of the present invention, the isolated nucleic acid encoding a polypeptide having an invertase activity is at least 80 %, at least 85 %, at least 90 %, at least 95 %, at least 98-100 % homologous to SEQ ID NOs:6 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

As used herein the terms "homology" or "homologous" refer to the resemblance between compared polypeptide sequences as determined from the identity (match) and similarity (amino acids of the same group) between amino acids which comprise these polypeptide sequences.

In addition, or alternatively this isolated nucleic acid is at least 80 %, at least 85 %, at least 90 %, at least 95 % identical with SEQ ID NOs:7 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to another preferred embodiment of the present invention, the isolated nucleic acid is hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 under moderate to stringent hybridization conditions suitable for polynucleotides longer than 200 base pairs.

Hybridization under moderate hybridization conditions is effected by a hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 1 x SSC and 0.1 % SDS and final wash at 50 or 55 °C whereas, hybridization under stringent hybridization conditions is effected by a hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 or 65 °C.

According to another preferred embodiment of the present invention, the isolated nucleic acid encodes a polypeptide which is as set forth in SEQ ID NOs:6 or 13 or portions thereof having the invertase activity.

5   These polypeptide sequences are designated herein as pLin5 (SEQ ID NOs:6) and eLin7 (SEQ ID NOs:13) which is encoded by the first two exons from the pLin5 gene and exons 3-6 of the eLin7 gene. These invertases include a secretion signal sequence and are expected to have high invertase activity under apoplastic environment conditions.

10   According to another preferred embodiment of the present invention the isolated nucleic acid is as set forth in SEQ ID NOs:7 or 11 or portions thereof.

According to another aspect of the present invention there is provided an isolated nucleic acid comprising a genomic, complementary

15   or composite polynucleotide sequence encoding a polypeptide having an invertase activity, the polypeptide is at least 80 %, at least 85 % at least 90 % at least 95 % at least 98-100 % homologous to SEQ ID NOs:6, 12 or 13, as determined using the BestFit software of the Wisconsin

sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

According to a preferred embodiment the isolated nucleic acid of this aspect of the present invention is hybridizable with SEQ ID NOs:1,

5 5, 7, 8, 9 or 11 under moderate to stringent hybridization conditions.

According to another preferred embodiment, the isolated nucleic acid of this aspect of the present invention is at least 80 %, at least 85 %, at least 90 %, at least 95 %, at least 98-100 % identical with SEQ ID NOs:7, 9 or 11 as determined using the BestFit software of the Wisconsin

10 sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

According to another preferred embodiment, the isolated nucleic acid of this aspect of the present invention the polypeptide encoded by

15 the isolated nucleic acid is as set forth in SEQ ID NOs:6, 12 or 13 or portions thereof.

The polypeptide encoded by SEQ ID NOs:6, or 13 are as mentioned above. SEQ ID NO:13 encodes a eLin7 invertase isoenzyme

which does not include a secretion signal peptide, but which is highly active in apoplastic conditions.

According to another preferred embodiment the isolated nucleic acid of this aspect of the present invention is as set forth in SEQ ID NOs:7, 9 or 11 or portions thereof.

According to another aspect of the present invention there is provided a nucleic acid construct including any of the isolated nucleic acid mentioned hereinabove.

The nucleic acid construct according to the present invention can be utilized to express the isolated nucleic acid within a plant, plant derived tissues, or plant cells either possessing a cell wall or not (protoplasts)

Thus, according to a preferred embodiment of the present invention, the nucleic acid construct further includes a promoter for regulating expression of the isolated nucleic acid in a sense or antisense orientation.

Numerous plant functional expression promoters and enhancers which can be either tissue specific, developmentally specific, constitutive

or induced and which can be utilized by the construct of the present invention, some examples are provided hereinunder.

As used herein in the specification and in the claims section that follows the phrase "plant promoter" includes a promoter which can direct

5   gene expression in plant cells (including DNA containing organelles). Such a promoter can be derived from a plant, bacterial, viral, fungal or animal origin. Such a promoter can be constitutive, i.e., capable of directing high level of gene expression in a plurality of plant tissues, tissue specific, i.e., capable of directing gene expression in a particular

10   plant tissue or tissues, inducible, i.e., capable of directing gene expression under a stimulus, or chimeric, i.e., formed of portions of at least two different promoters.

Thus, the plant promoter employed can be a constitutive promoter, a tissue specific promoter, an inducible promoter or a chimeric promoter.

15   Examples of constitutive plant promoters include, without being limited to, CaMV35S and CaMV19S promoters, FMV34S promoter, sugarcane bacilliform badnavirus promoter, CsVMV promoter, *Arabidopsis* ACT2/ACT8 actin promoter, *Arabidopsis* ubiquitin UBQ1 promoter, barley leaf thionin BTH6 promoter, and rice actin promoter.

Examples of tissue specific promoters include, without being limited to, bean phaseolin storage protein promoter, DLEC promoter, PHSβ promoter, zein storage protein promoter, conglutin gamma promoter from soybean, AT2S1 gene promoter, ACT11 actin promoter

5   from *Arabidopsis*, napA promoter from *Brassica napus* and potato patatin gene promoter.

The inducible promoter is a promoter induced by a specific stimuli such as stress conditions comprising, for example, light, temperature, chemicals, drought, high salinity, osmotic shock, oxidant conditions or in

10   case of pathogenicity and include, without being limited to, the light-inducible promoter derived from the pea rbcS gene, the promoter from the alfalfa rbcS gene, the promoters DRE, MYC and MYB active in drought; the promoters INT, INPS, prxEa, Ha hsp17.7G4 and RD21 active in high salinity and osmotic stress, and the promoters hsr203J and

15   str246C active in pathogenic stress.

The construct according to the present invention preferably further includes an appropriate selectable marker such as for example an antibiotic resistance gene. In a more preferred embodiment according to

the present invention the construct further includes an origin of replication.

The construct according to the present invention can be a shuttle vector, which can propagate both in *E. coli* (wherein the construct comprises an appropriate selectable marker and origin of replication) and be compatible for propagation in cells, or integration in the genome, of a plant. The construct according to this aspect of the present invention can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome.

There are various methods of introducing nucleic acid constructs into both monocotyledonous and dicotyledenous plants (Potrykus, I., Annu. Rev. Plant. Physiol., Plant. Mol. Biol. (1991) 42:205-225; Shimamoto *et al.*, Nature (1989) 338:274-276). Such methods rely on either stable integration of the nucleic acid construct or a portion thereof into the genome of the plant, or on transient expression of the nucleic acid construct in which case these sequences are not inherited by a progeny of the plant

There are two principle methods of effecting stable genomic integration of exogenous nucleic acid sequences such as those included

within the nucleic acid construct of the present invention into plant genomes:

(i)   *Agrobacterium*-mediated gene transfer: Klee *et al.* (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.

(ii) direct DNA uptake: Paszkowski *et al.*, in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. *et al.* (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang *et al.* Plant Cell Rep. (1988) 7:379-384. Fromm *et al.* Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein *et al.* Bio/Technology (1988) 6:559-563; McCabe *et al.* Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990)

79:206-209; by the use of micropipette systems: Neuhaus *et al.*, Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; or by the direct incubation of DNA with germinating pollen, DeWet *et al.* in Experimental Manipulation of Ovule

5   Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels, W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

The *Agrobacterium* system includes the use of plasmid vectors that contain defined DNA segments that integrate into the plant genomic

10   DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the *Agrobacterium* delivery system. A widely used approach is the leaf disc procedure which can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation. Horsch *et al.* in Plant Molecular Biology Manual A5,

15   Kluwer Academic Publishers, Dordrecht (1988) p. 1-9. A supplementary approach employs the *Agrobacterium* delivery system in combination with vacuum infiltration. The *Agrobacterium* system is especially viable in the creation of transgenic dicotyledenous plants.

There are various methods of direct DNA transfer into plant cells. In electroporation, protoplasts are briefly exposed to a strong electric field. In microinjection, the DNA is mechanically injected directly into the cells using very small micropipettes. In microparticle bombardment,

5 the DNA is adsorbed on microprojectiles such as magnesium sulfate crystals, tungsten particles or gold particles, and the microprojectiles are physically accelerated into cells or plant tissues.

Following transformation plant propagation is exercised. The most common method of plant propagation is by seed. Regeneration by

10 seed propagation, however, has the deficiency that due to heterozygosity there is a lack of uniformity in the crop, since seeds are produced by plants according to the genetic variances governed by Mendelian rules. Basically, each seed is genetically different and each will grow with its own specific traits. Therefore, it is preferred that the transformed plant

15 be produced such that the regenerated plant has the identical traits and characteristics of the parent transgenic plant. Therefore, it is preferred that the transformed plant be regenerated by micropropagation which provides a rapid, consistent reproduction of the transformed plants.

Transient expression methods which can be utilized for transiently expressing the isolated nucleic acid included within the nucleic acid construct of the present invention include, but are not limited to, microinjection and bombardment as described above but under

5   conditions which favor transient expression, and viral mediated expression wherein a packaged or unpackaged recombinant virus vector including the nucleic acid construct is utilized to infect plant tissues or cells such that a propargating recombinant virus established therein expresses the non-viral nucleic acid sequence.

10   Viruses that have been shown to be useful for the transformation of plant hosts include CaV, TMV and BV. Transformation of plants using plant viruses is described in U.S. Pat. No. 4,855,237 (BGV), EP-A 67,553 (TMV), Japanese Published Application No. 63-14693 (TMV), EPA 194,809 (BV), EPA 278,667 (BV); and Gluzman, Y. *et al.*,

15   Communications in Molecular Biology: Viral Vectors, Cold Spring Harbor Laboratory, New York, pp. 172-189 (1988). Pseudovirus particles for use in expressing foreign DNA in many hosts, including plants, is described in WO 87/06261.

Construction of plant RNA viruses for the introduction and expression of non-viral exogenous nucleic acid sequences in plants is demonstrated by the above references as well as by Dawson, W. O. *et al.*, Virology (1989) 172:285-292; Takamatsu *et al.* EMBO J. (1987) 6:307-311; French *et al.* Science (1986) 231:1294-1297; and Takamatsu *et al.* FEBS Letters (1990) 269:73-76.

When the virus is a DNA virus, the constructions can be made to the virus itself. Alternatively, the virus can first be cloned into a bacterial plasmid for ease of constructing the desired viral vector with the foreign DNA. The virus can then be excised from the plasmid. If the virus is a DNA virus, a bacterial origin of replication can be attached to the viral DNA, which is then replicated by the bacteria. Transcription and translation of this DNA will produce the coat protein which will encapsidate the viral DNA. If the virus is an RNA virus, the virus is generally cloned as a cDNA and inserted into a plasmid. The plasmid is then used to make all of the constructions. The RNA virus is then produced by transcribing the viral sequence of the plasmid and translation of the viral genes to produce the coat protein(s) which encapsidate the viral RNA.

Construction of plant RNA viruses for the introduction and expression in plants of non-viral exogenous nucleic acid sequences such as those included in the construct of the present invention is demonstrated by the above references as well as in U.S. Pat. No.

5   5,316,931.

In one embodiment, a plant viral nucleic acid is provided in which the native coat protein coding sequence has been deleted from a viral nucleic acid, a non-native plant viral coat protein coding sequence and a non-native promoter, preferably the subgenomic promoter of the non-

10   native coat protein coding sequence, capable of expression in the plant host, packaging of the recombinant plant viral nucleic acid, and ensuring a systemic infection of the host by the recombinant plant viral nucleic acid, has been inserted. Alternatively, the coat protein gene may be inactivated by insertion of the non-native nucleic acid sequence within it,

15   such that a protein is produced. The recombinant plant viral nucleic acid may contain one or more additional non-native subgenomic promoters. Each non-native subgenomic promoter is capable of transcribing or expressing adjacent genes or nucleic acid sequences in the plant host and incapable of recombination with each other and with native subgenomic

promoters. Non-native (foreign) nucleic acid sequences may be inserted adjacent the native plant viral subgenomic promoter or the native and a non-native plant viral subgenomic promoters if more than one nucleic acid sequence is included. The non-native nucleic acid sequences are transcribed or expressed in the host plant under control of the subgenomic promoter to produce the desired products.

In a second embodiment, a recombinant plant viral nucleic acid is provided as in the first embodiment except that the native coat protein coding sequence is placed adjacent one of the non-native coat protein subgenomic promoters instead of a non-native coat protein coding sequence.

In a third embodiment, a recombinant plant viral nucleic acid is provided in which the native coat protein gene is adjacent its subgenomic promoter and one or more non-native subgenomic promoters have been inserted into the viral nucleic acid. The inserted non-native subgenomic promoters are capable of transcribing or expressing adjacent genes in a plant host and are incapable of recombination with each other and with native subgenomic promoters. Non-native nucleic acid sequences may be inserted adjacent the non-native subgenomic plant viral promoters

such that said sequences are transcribed or expressed in the host plant under control of the subgenomic promoters to produce the desired product.

In a fourth embodiment, a recombinant plant viral nucleic acid is provided as in the third embodiment except that the native coat protein coding sequence is replaced by a non-native coat protein coding sequence.

The viral vectors are encapsidated by the coat proteins encoded by the recombinant plant viral nucleic acid to produce a recombinant plant virus. The recombinant plant viral nucleic acid or recombinant plant virus is used to infect appropriate host plants. The recombinant plant viral nucleic acid is capable of replication in the host, systemic spread in the host, and transcription or expression of foreign gene(s) in the host to produce the desired protein.

Thus, according to a preferred embodiment of the present invention the polynucleotide or nucleic acid molecule of the present invention further includes one or more sequence elements, such as, but not limited to, a nucleic acid sequence encoding a transit peptide, an origin of replication for propagation in bacterial cells, at least one

sequence element for integration into a plant's genome, a polyadenylation recognition sequence, a transcription termination signal, a sequence encoding a translation start site, a sequence encoding a translation stop site, plant RNA virus derived sequences, plant DNA virus derived sequences, tumor inducing (Ti) plasmid derived sequences and a transposable element derived sequence.

According to another aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing in the plant tissue a polypeptide having invertase activity, wherein the polypeptide is at least 80 %, at least 85 %, at least 90 % at least 95 %, at least 98-100 % homologous to SEQ ID NOs:6, 12 or 13 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

The polypeptide according to this aspect of the present invention is preferably encoded by a polynucleotide which is hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 or a portion thereof under mild or stringent hybridization conditions as described above.

To effect expression, this polynucleotide sequence is preferably included in nucleic acid construct which also includes a promoter and selection markers as described hereinabove.

It will be appreciated that any of the transformation methods described hereinabove can be used to transform a plant or plant tissues with the above described construct, such that expression of the isolated nucleic acid according to any aspect of the present invention is effected.

According to another aspect of the present invention, there is provided an isolated regulatory element comprising a polynucleotide at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 85 %, at least 90 %, at least 95 % at least 98-100 % identical with SEQ ID NO:4 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

Additionally or alternatively this polynucleotide is hybridizable with SEQ ID NO:4 under mild to moderate hybridization conditions.

Hybridization under mild hybridization conditions is effected by a hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 %

SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 2 x SSC and 0.1 % SDS and final wash at 48 °C.

The regulatory element encoded by the polynucleotide according to this aspect of the present invention is further described in Example 5 of the Examples section. This novel regulatory element which is unique to *L. Pennellii* is associated the high brix value found in *L. pennelli* fruit.

As such stable genetic integration of the nucleic acid sequence of this regulatory element into the same chromosomal site (described in Example 5) of genetically similar solanaceae plants such as but not limited to, pepper and potato will increase their fruit monosaccharide content as compared to non-transformed plants.

Thus according to another aspect of the present invention there is provided a method of increasing a level of a monosaccharide in a tissue of a solanaceae plant, the method comprising the step of integrating into a genome of the solanaceae plant a polynucleotide including a nucleic acid sequence as set forth in SEQ ID NO:4, wherein said polynucleotide is integrated into a specific site of chromosome 9 of the solanaceae plant via homologous recombination.

To effect homologous recombination the regulatory element is preferably included in a nucleic acid construct. The nucleic acid construct according to this aspect of the present invention further includes positive and negative selection markers and may therefore be employed for

5   selecting for homologous recombination events, such as for example, homologous recombination employed in knock-in procedures. Numerous examples to methods and strategies for effecting site directed homologous recombination in plants exist in the art as such no further detail is necessary herein. One ordinarily skilled in the art can readily

10   design a knock-in constructs including both positive and negative selection genes for efficiently selecting transformed plant cells that underwent a homologous recombination event with the construct. Such cells can then be cultured into a plant as described hereinabove.

According to another aspect of the present invention there is

15   provided a method for determining whether fruits to be produced from solanaceae seeds or solanaceae seedling will contain an amount of monosaccharides above a predetermined threshold.

The method according to this aspect of the present invention is effected by detecting the presence or absence of the regulatory nucleic

acid sequence (SEQ ID NO:4) in genomic DNA derived from the solanaceae seeds or solanaceae seedling.

Since the regulatory element encoded by SEQ ID NO:4 serves as a marker for the high Brix trait isolated herein from *L. pennellii*, detection of this sequence in genomic DNA derived from seeds or immature seedlings can enable the determination of the monosaccharide content of the fruit to be produced from mature plants grown from these seeds or seedlings.

Thus the present invention describes novel genes encoding apoplastic invertase isoenzymes which function either in combination or individually in elevating the monosaccharide of plants expressing same.

Furthermore the present invention describes a novel regulatory element which is unique to *L. pennellii* and which is associated with the high brix trait thereof. Preliminary data presented in the Examples section which follows suggests that this regulatory element functions in either regulating the expression of an invertase gene located downstream thereto, or in directing alternative splicing events which generate a chimeric invertase transcript.

A promoter sequence controlling the expression of pLin5 is also within the scope of the present invention. Such a promoter resides upstream to pLin5 and its sequence is included in SEQ ID NO:1 (nucleotides 1-4849).

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example,

"Molecular Cloning: A laboratory Manual" Sambrook et al., (1989);

"Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M.,

ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology",

John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical

5   Guide to Molecular Cloning", John Wiley & Sons, New York (1988);

Watson et al., "Recombinant DNA", Scientific American Books, New

York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual

Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York

(1998); "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J.

10   E., ed. (1994); "Oligonucleotide Synthesis" Gait, M. J., ed. (1984);

"Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds.

(1985); "Transcription and Translation" Hames, B. D., and Higgins S. J.,

eds. (1984); "A Practical Guide to Molecular Cloning" Perbal, B., (1984)

and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR

15   Protocols: A Guide To Methods And Applications", Academic Press, San

Diego, CA (1990); Marshak et al., "Strategies for Protein Purification

and Characterization - A Laboratory Course Manual" CSHL Press

(1996); "An introduction to genetic analysis"-third edition, Suzuki et al.,

1986 and "Molecular Dissection of Complex Traits, Paterson AH 1998;

all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information

5 contained therein is incorporated herein by reference.

EXAMPLE 1

In previously published results (Eshed and Zamir, 1995, *ibid*) an *L. pennellii* introgression line (IL) population was designed in order to generate QTL-NILs. This IL population consisted of 50 lines, each

10 containing a single homozygous restriction fragment length polymorphism (RFLP)-defined wild-species chromosome segment. Together these lines provided complete coverage of the tomato genome and a set of nearly isogenic lines (NILs) to their recurrent parent, the processing-tomato cultivar M82 (Rick et al., TGRC stock lists, *Rep.*

15 *Tom. Genet. Coop.*, 45, 53, 1995) (Figure 1). The genetic assumption underlying the identification of QTL using the NILs was that any phenotypic difference between an IL and its nearly isogenic control plant is due to a QTL that resides on the chromosome segment introgressed from *L. pennellii*. The minimum number of $p<0.05$-significant QTL

affecting a trait in the ILs was calculated on the basis of the following assumptions: (i) each IL affecting a quantitative trait carries only a single QTL; and (ii) two overlapping introgressions with a significant effect on a trait (in the same direction relative to the control) carry the same QTL.

5   Therefore, in the ILs, the maximum number of detectable QTLs is approximately 30.   Despite this limitation, twice as many QTLs responsible for fruit mass (FM) were identified as compared to the previous populations (see Table 1 below). The sensitivity of the ILs in identifying QTLs was even more pronounced for brix (B), where two to

10   six times as many QTLs were identified as compared to the other populations.

Table 1
The number of significant effects (p<0.05) of wild species QTLs on FM and B

Species	population structure	population size	No. of FM-QTLs	No. of B-QTLs	Reference
L. chmielewskii	BC1	237	6	4	Tanksley et al. *Genetics*, 232, 1141, 1992
L. cheesmanii	F2	350	7	4	Paterson et al *Genetics*, 127, 181, 1991
L. pimpinellifolium	BC1	257	7	3	Grandillo et al *Theor. Appl. Genet.*, 90, 225, 1996
L. cheesmanii	RI	97(6 reps.)	12	14	Goldman et al *Theor. Appl. Genet.*, 90, 925, 1995
L. pennellii	IL	50 (6 reps.)	18	23	Eshed and Zamir 1995, *ibid*

15

Using the *L. pennellii* ILs, QTLs were mapped to various chromosome segments originating from the wild species. However the effects associated with an introgressed segment could be due to the existence of one or more loci.   A 60-cM segment on the long arm of chromosome 2 was responsible for a 60 % reduction in FM (in homozygotes) relative to the control, M82.   This chromosomal region apparently harbors QTLs responsible for FM, which are common to a number of wild tomato species (Alpert et al. Theor. Appl. Genet., 91, 994, 1995).

Fine-mapping analysis of recombinant lines for that region identified three linked loci with a similar effect on FM; two of which were placed on a 3 cM interval.   Finer mapping may reveal additional FM QTL in these regions. Quantitative effects which appear to be associated with a single locus were inferred from cases of rare transgressive segregation. Using the ILs, 18 QTLs responsible for FM were identified but in only two cases (IL7-5 and IL12-1-1 with introgression sizes of 15 and 4 cM, respectively) alleles of the small-fruited wild species were associated with larger fruits.   These effects

were consistent in trials conducted in different years and genetic constitutions (Eshed and Zamir, 1996, *ibid*).

Several features of the IL population contributed to its efficiency in detecting QTLs, even in cases when only a few replicates of each genotype were evaluated.

(i)   The lines contained single RFLP-defined introgressions, some of which produce effects of relatively large magnitude in which most of the phenotypic variation between the NILs is associated with the introgressed segment.

(ii)   The permanent nature of the lines enabled testing of the introgression effects in different years. The results obtained showed high reproducibility of the effects of the QTL which were mapped to the different introgressed chromosome segments.

(iii)   Elimination of the "overshadowing effect" of major QTLs enabled to detect minor QTLs (a major QTL contributes to large phenotypic variation, thereby masking the effects of other QTLs segregating in the same population)

(iv)   Elimination of epistatic interactions between unlinked QTLs.

(v)   The simple statistical procedure relied on comparison with a common control and is therefore less affected by experimental error.

Gene actions revealed by QTL studies

A gene action of the QTL detected was determined using the IL population described above by comparing the homozygous ILs to hybrids of the ILs with the recurrent parent.

The FM and brix qualities were determined by QTLs which were intermediates between additivity and dominance.   This mode of inheritance is in agreement with results obtained by analysis of an F2 generation (Paterson Genetics, 127, 181, 1991).   In contrast, fruit yield (Y) was strongly associated with overdominance, whereby some of the heterozygous ILs had higher values relative to their corresponding homozygous parents.

Detailed mapping analysis of a chromosome 1 introgression which showed overdominance for Y suggested the existence of two *cis* loci with opposing effects. This result was therefore consistent with the pseudo-overdominance model for heterosis (Crow *Heterosis*, Gowen, J. W., Eds., Iowa State College Press, Ames, IA, 1952). However for the other heterotic introgressions, including *dw-1*, the issue of the mode of gene

action for heterosis is still unresolved. It is interesting to note that the wild species used for the tomato mapping studies were highly inferior to the cultivated variety with respect to Y, yet chromosome segments from these species contribute to the increased Y of commercially grown

5   varieties. This transgressive segregation is frequent for Y and for seedling morphological traits, whereas for FM and brix, transgression was rare (De-Vicente et al Genetics, 134, 585, 1993).

Reproducibility of the effects of an identified QTL

Mendelian factors underlying quantitative traits in an interspecific

10   tomato cross were compared in F2 and F3 generations of the same population (Paterson Genetics, 127, 181, 1991). Of 11 FM QTL identified in both generations in a trial conducted in California, six were significant both in F2 and F3. Of the five B QTLs, two were significant in both generations. Differences between generations can result from

15   interactions with the environment and/or may indicate that the resolution power of such populations is limited to QTLs with large effects. In contrast, of 33 yield-associated QTLs identified in a two-year trial of selected ILs, 28 were significant in both experiments (Eshed and Zamir 1996, *ibid*).

Association between QTL-NILs and the introgressed segment

The use of the *L. pennellii* ILs to identify QTLs is based on RFLP results which indicated that each line contains a single wild-species introgression. However, some of the lines may include small unidentified introgressions, and these segments may be responsible for the observed phenotypic effects. To test whether the difference between the IL and its nearly isogenic control lies solely in the introgressed segment, a simple experiment was performed using eight selected ILs. An F2 resulting from a cross between each IL and M82 was subjected to RFLP analysis, and plants homozygous for the cultivated-tomato chromosome segment were compared quantitatively to M82. In no case were any differences detected, indicating that the observed phenotypic differences (which were verified using the plants carrying the *L. pennellii* introgressions) are due to the mapped chromosome segment.

EXAMPLE 2

Epistatic interactions

The study described in Example 1 (Eshed and Zamir, 1995, *ibid*) served as a basis for testing epistatic interactions between QTLs. Thus, 10 ILs were selected, some of which include QTLs that affect the

measured traits in the heterozygous condition in various directions relative to the control the results obtained were reported.

The ten homozygous ILs were crossed in a half diallele mode and the phenotypic values of the 45 double heterozygotes were compared to the respective single heterozygous ILs and M82. The results which were previously reported by Eshed and Zamir (1996, *ibid*) indicate that QTL epistasis is prevalent and is generally less than additive.

Phenotype of the selected single introgression ILHs:

In the complete IL population composed of 50 introgression line hybrids (ILHs) which was analyzed for five yield-associated traits, 81 of the 250 ILH x trait combinations (32 %) were significantly different from the isogenic controls ($p < 0.05$). For the subset of the 10 ILHs selected for the interaction study, 30 of the 50 combinations (60 %) differed significantly from the control (Figure 2). This comparison indicates that the 10 ILHs were enriched for QTLs affecting the measured traits.

In this previously reported study, of the 10 ILHs (using the same experimental error), 28 of the 30 significant effects were consistent between the two experiments (Table 2 below; Y for ILH1-4 and BY for ILH2-6-1 were not significantly different from the control).

Table 2
Mean phenotypic values of M82 and the IL hybrids heterozygous for single introgressions

Genotype	Introgressed region 'a'	Number of replicates	plant weight (kg)	fruit mass (g)	brix (0)	Yield (kg)	brix x yield (g)
M82	none	79	1.82± 0.44	56.1± 4.9	4.54± 0.40	9.18± 1.54	417± 82
ILH1-1[b]	1(CT233-TG71; 58 cM)	26	3.56± 0.84*	48.6± 6.5*	5.23± 0.48*	11.15± 2.34*	580± 114*
ILH1-4	1(TG245-TG259; 35 cM)	23	2.10± 0.44	58.1± 5.4	5.16± 0.39*	9.84± 1.60	507± 83
ILH2-1	2(R45S- TG276; 16cM)	26	1.27± 0.32*	52.4± 5.4*	4.01± 0.33*	7.19± 1.64*	289± 72*
ILH2-6-1[c]	2(TG91- CT59; 14cM	26	2.68± 0.46*	35.2± 4.5*	5.30± 0.44*	8.95± 1.81	474± 99
ILH5-4	5(TG351- TG413; 16cM)	25	2.62± 0.59*	57.9± 7.1	5.07± 0.31*	10.85± 2.34*	551± 127*
ILH7-5	7(TG61- TG131A; 15 cM)	26	2.46± 0.46*	61.5± 6.3*	4.83± 0.33*	10.52± 1.45*	509± 87*
ILH9-2-5[c]	9(CT283A- TG10;9cM)	25	2.09± 0.47	51.7± 6.3*	5.52± 0.26*	9.58± 1.92	532± 122*
ILH10-1	10(TG230- TG28; 37cM)	24	1.84± 0.33	46.5± 5.5*	5.11± 0.31*	_8.38±_ _1.60_	_428±_ _81_
ILH11-1	11(TG497- TG523 27cM)	26	2.06± 0.47	47.5± 3.6*	4.79± 0.41	_8.50_ _±1.65_	_406±_ _73_
ILH12-1-1[c]	12 (TG180- ACO- 1; 4cM)	27	1.81± 0.32	63.3± 5.4*	4.70± 0.37	8.96 ±1.21	422± 69

Mean phenotypic values and standard deviations of M82 and the ILH that participated in the diallele crosses. All means were compared to M82 and the ones marked with * are significantly different (Dunnet's t-test, $p<0.05$). Underlined mean values indicate a significant interaction with year (1993 vs., 1995; $0.01<p<0.05$).

'a' - The introgressed regions in the ILHs is indicated by chromosome number, the markers flanking the introgression and its size in cM according to Tanskley et al. (1992) Genetics 132:1141-1160.

[b] - ILH - Hybrid of ILs crossed with M82.

[c] - Interaction with year was based on unpublished results from a 1994 trial.

The effects of ILH7-5 on PW, FM and brix were found to be significant as compared to other previously reported studies. this significance was probably due to the larger number of replicants tested (25 as compared to 6 in previous studies). Significant ILH by year interactions ($p<0.05$) were detected for four of the 50 comparisons (Table

2). These four comparisons were not significantly different from the control in either of the years. These results indicate a high overall reproducibility of the experimental system in different years of growth.

Interactions between unlinked introgressions:

5   The null hypothesis for the interaction analysis was complete additivity of the effects of the single introgression ILHs. Any significant deviation from complete additivity was considered as epistasis (Figure 3).

For example, ILH1-1 increased PW by 95 % compared to M82; ILH12-1-1 reduced PW by 1 % compared to M82. The expected phenotype for the hybrid between the two homozygous ILs (IL1-1 and

10   IL12-1-1) is a 94% increase in PW relative to M82. The observed PW for the hybrid heterozygous for the two introgressions was 76 % higher than M82, indicating a significant interaction ($p < 0.05$).

Of the 225 possible interactions (45 hybrids x five traits) 59, 28

15   and 12 were significant at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level respectively (Figure 3). These values are much higher than that expected by chance alone.

To further characterize the nature of the interactions, the double-heterozygous combinations were divided into four groups based on the performance of the single ILHs (Table 3 below)

Table 3
Frequency of significant interactions (p<0.05) between unlinked L. pennellii introgressions

Interacting QTL types[a]	Plant weight	Fruit mass	brix	Total fruit yield	brix×yield	Sum
Sig-Sig (same direction)	3[b]/6[c]	8/16	12/21	1/3	5/15	29/61
Sig-Sig (opposite direction)	2/4	1/12	3/7	0/3	1/6	7/32
Sig-NonSig	5/25	2/16	4/16	5/24	2/21	18/102
NonSig-NonSig	1/10	0/1	0/1	3/15	1/3	5/30
Sum	11/45	11/45	19/45	9/45	9/45	59/225

[a] QTLs were classified according to the significance and the direction of their effects relative to M82.
[b] Number of significant interactions.
[c] Number of tested combinations of two *L. pennellii* introgressions.

As is shown by Table 3, of 61 tested introgressions between significant QTLs (same direction), 29 (48 %) were significant (p<0.05) indicating that the interactions between two significant QTLs of *L. pennellii* affect a trait in the same direction.

Among 32 introgressions between significant (opposite) QTLs, seven (22 %) significant interactions were detected indicating that the interaction between two significant QTLs of *L. pennellii* affect the trait in opposite directions. Six of these interactions involved crosses with IL2-1

for PW, B and BY. The IL2-1 line carries the pleiotropic QTL which affected all of these traits. The seventh interaction in this group involved that of IL12-1-1 with IL-10-1 for FM, where IL12-1-1 showed marked transgressive segregation for this trait (Table 2).

Among 102 introgressions between significant and non-significant QTLs 18 (18 %) interactions were significant.

Among the 30 introgressions between non-significant QTLs, five (17 %) significant interactions were found. Overall, 26 % (59/225) of the various *L. pennellii* introgressions showed significant interactions and the proportion of epistatic effects was highest for significant same direction QTLs.

To search for general trends in the interaction of QTLs, the observed values of the 45 double-heterozygous hybrids were plotted against their expected values (Figure 4).

For all five traits highly significant linear regressions were found, indicating the overall additivity of the effects of the independent introgressions. Assuming complete additivity between the effects of the combined individual introgressions one would expect a regression with a slope of 1. The slopes of the lines for the five traits were significantly

lower than 1 (ranging from 0.71-0.79), indicating average combined effects which are less than additive.

To further examine the less than additive trend revealed by the regression analysis, only the cases of epistasis between significant QTL affecting the traits in the same direction were examined irrespective of whether the QTL originated from *L. pennellii* or *L. esculentum*.

Twenty-nine epistatic interactions between *L. pennellii* introgressions were detected. In all cases, the observed means for the double heterozygous ILHs were significantly lower than the values expected on the basis of an assumption of complete additivity. Seven of the interactions of QTL affecting the trait in the same direction involved *L. pennellii* introgressions with *L. esculentum* alleles. In these cases (row 2 of Table 3), the *L. pennellii* introgressions affected the trait in an opposite manner to that expected according to the parental phenotype (transgressive QTL). Six of the seven interactions were less than additive; the only exception was PW for the hybrid of IL1-1 x IL2-1. In this case the double heterozygous hybrid for the QTL acting in the same direction (ILH1-1) showed a higher mean value than the sum of the two

independent QTL (M82 and IL2-1 x IL1-1). Overall, 35 of the 36 interactions (97 %) showed less than additive interactions.

Interactions between linked QTL (chromosome 2):

Twelve homozygous ILs with different introgression sizes in chromosome 2 were evaluated for FM and B. Since 10 of these lines were previously tested (Eshed and Zamir 1995, *ibid*) and no significant interactions between year and IL were detected, the results from the two years were pooled. Based on the overlapping recombined chromosome segments and the phenotypic value of each of the ILs, two B QTL and three FM QTL, responsible for a similar reduction in fruit mass, were mapped (Figure 5-6). After determining the positions of these QTL, the lines were classified according to their postulated genotypes (Table 4).

Table 4
Interactions of linked QTLs responsible for brix and fruit mass

Genotypic group[a]	Mean brix (B) in Brix units	Mean brix Δ % from M82	P value of interaction
No QTL[b]	4.47	-0.2	
B2-1	5.00	11.7	
B2-2	4.98	11.6	
B2-1/2-2	5.37	19.9	0.03
Genotypic group[a]	Mean Fruit mass (FM) in grams	Mean FM Δ % from M82	P value of interaction
No QTL[b]	59.5	0.6	
Fm2-1	43.0	-27.3	
Fm2-2	41.0	-30.7	
Fm2-3	42.7	-27.8	
Fm2-1/2-2	30.7	-48.2	0.009
Fm2-2-/2-3	28.0	-52.7	0.03
Fm2-1/2-2/2-3	21.0	-64.6	<0.0001/<0.0001[c]

[a] genotypic groups were pooled on the basis of the fine mapping analysis presented in Figure 5.

b M82 was included in this group, which includes lines without an L. pennellii QTL which affects this trait.

c The two tested interactions were Fm2-1×Fm2-2/2-3 and Fm2-3×Fm2-1/2-2

Epistasis for B and FM was tested by comparing the means of the pooled genotypic groups. The single interaction for B was significant and the sum of the effects of the single QTL was higher than the mean value of the lines carrying both QTL. The four different tests for FM QTL interactions were significant: two of them examined the combined action of a single QTL and two examined a single QTL and the remaining pair. The average diminishing effect for two QTL was 8.5 % compared to 16.2 % for interactions involving the three QTL (Table 3). This result suggests that the effect of the less than additive epistasis is increased (i.e. the effects are further diminished) when more QTL are involved.

The nearly isogenic nature of the IL population utilized by this study allows the identification of twice as many QTL affecting FM and B as in other interspecific studies in tomato (Eshed and Zamir, 1995, *ibid*). The isogenic nature of the IL population is also responsible for the ability to determine epistasis of QTL through the design of experiments with balanced representation of the different genotypes. Nearly isogenic lines were previously demonstrated to be very efficient for the detection of epistasis of QTL in *Drosophila* (Long *et al.* 1995, Genetics 139:1273-

1291) and maize (Doebley *et al.* 1995, Genetics 141: 333-346). In conventional segregating populations (F2/F3, BC and recombinant inbreds) all the QTLs which affect the trait are segregating QTLs. Assuming that the less than additive mode of epistasis detected in this study is common to other tomato crosses, this interaction would reduce the effect of individual QTLs. As a consequence, the number of significant QTLs would be underestimated. Less than additive interactions among QTL ensure that the "loss" of an allele affecting a fitness trait will have a minimal effect on the phenotype and that canalization will be achieved.

Contrary to past QTL mapping studies that uncovered little evidence for epistasis, QTL epistasis is an important component in determining the phenotypic value for traits showing continuous variation (Table 3). Of the 93 combinations of pairs of significant QTLs, 39 % were epistatic at a significance level of $p<0.05$. Moreover, a higher frequency of epistasis than expected by chance alone was detected for *L. pennellii* chromosome segments that individually did not affect the traits (17 %).

Thus, the prevalence of epistasis uncovered by this study is consistent with the numerous classical studies of quantitative traits and breeding that show significant overall epistatic effects for quantitative traits detected through biometrical genetics.

EXAMPLE 3

Separating the positive trait for high brix value from the negative traits of percentage green fruit yield and internodes length through marker assisted selection

As is described in Examples 1 and 2, the hybrid plants obtained from introgressing *L. pennellii* into a *L. esculentum* genetic background detected numerous QTLs associated with traits such as brix (B) and fruit mass (FM). However, these studies failed to isolate the QTL associated with brix from other QTLs which are associated with negative traits such as high percentage of green fruit yield and long internodes.

As such, while reducing the present invention to practice a hybrid plant (IL9-2-5) resultant from these studies was further introgressed into the genetic background of an *L. esculentum* cultivated tomato variety (M82) in efforts to isolate the QTL associated with brix from other QTLs responsible for these negative traits which are present in IL9-2-5, to

thereby obtain a plant line bearing fruits characterized by a high sugar content (high brix value) while being otherwise similar in phenotype to a cultivated tomato.

To estimate the phenotypic variation associated with high brix value, near isogenic plants derived from self crossing of M82 and IL9-2-5 and hybrids generated from crossing M82 and IL9-2-5, were evaluated over a three year period. Figure 7 presents the means of the tested genotypes for total soluble-solids (brix, B), plant weight (PW) and fruit mass (FM).

The 9-2-5 (chromosome 9) introgression was responsible for a significant reduction (10 %) in FM in 1995 while in the following years its effects were not significant. The effect of the introgression on B was consistent between the different years; the introgression significantly increased B from 20 to 32 percent relative to the control showing partial dominance (d/a=0.64). In 1995, the introgression increased PW by ten percent compared to 70 percent in 1997; yet, the effect of the introgression on B was similar in these two years, indicating that PW is not involved in the major pathway affecting B. Hybrid high brix value plants that carried the introgression were more vegetative, with longer

internodes and the ripening of the fruit lasted a longer period (late variety) and as such are of little commercial value.

In order to generate hybrids characterized by a uniform and early ripening, a good cover of the fruit and a high brix value, which hybrids

5 are of high commercial value, it was decided that further narrowing of the 9-2-5 introgression chromosomal region (9 cM) must be effected in order to isolate the brix QTL.

In order to verify if the increase in brix and the negative effects described above which are associated with the 9-2-5 introgression are

10 due to linkage drag or are simply pleiotropic effects of the QTL, sub-lines of IL9-2-5 (IL9-2-6 and IL9-2-7) were generated by selfing the IL9-2-5 hybrid and screening for recombinants in the introgression. IL9-2-6 and IL9-2-7 carried the south (in direction of the centromere) and north (in direction of a telomere) part of the introgression, respectively (Figure

15 8a). Plants of M82 and hybrids generated from crossing M82 with the IL9-2-5, IL9-2-6 and IL9-2-7 plant lines (termed ILH9-2-5, ILH9-2-6 and ILH9-2-7, respectively) were planted in a commercial stand and evaluated for B, FM, vegetation and % of green fruit yield as a parameter for the uniformity of the ripening. Figure 8b presents the mean effects of

the tested hybrids as is compared to the control tomato plant M82. The short introgressions of IL9-2-7 showed the "negative" phenotype of IL9-2-5 with high vegetation, longer internodes and late maturity, but had no significant effect on B. IL9-2-6 had a significant increasing effect on B

5 with a reduced vegetation and an early and uniform ripening. The three hybrids had no significant effects on the FM.

Thus, these results place the brix QTL in the south part of the 9-2-5 introgression (Figure 8a). A hybrid plant (ILH9-2-6) generated from introgressing IL9-2-6 in the M82 genetic background is characterized by

10 fruit having an increased sugar content (B) similar to that of the IL9-2-5 hybrid plant line, without the undesired traits found in IL9-2-5 which are generated by genes situated in the northern part of the 9-2-5 introgression (9-2-7).

EXAMPLE 4

15 The study described in Example 3 which was conducted as part of the present invention and previously published studies described in Examples 1 and 2, were performed in a genetic background of determinate tomato lines that were specifically developed for the processing tomato industry (M82). These plants are suitable for "once

over" machine harvest due to homozygosity for the recessive mutation *sp* (*self pruning*) which modifies the developmental program of the shoot such that growth is terminated after the production of two consecutive inflorescences.

5   The wild species (green-fruited) and greenhouse cultivated tomatoes are indeterminate (Sp^+) where the shoot follows a uniform developmental program of three leaves and an inflorescence throughout the growth (Pnueli et al. 1998, Development 125:1979-1989). Indeterminate greenhouse tomatoes require different agricultural

10   practices as is compared to determinate varieties and therefore constitute a fundamentally different genetic background to test the effect of the brix QTL.

M82 and IL9-2-5 were crossed with an indeterminate greenhouse line (202) and the two nearly isogenic indeterminate hybrids were grown

15   in the greenhouse and evaluated for B.   The introgression was responsible for a 40 percent increase in B with a separation of the values into discrete groups (Figure 9). This result gave a motivation to develop NILs for the chromosome 9 introgression in the genetic background of line 202.

The initial material for the introduction of the brix QTL into indeterminate background was the IL9-2-4 introgression line (Figure 10). This introgression extends to the south of the chromosome beyond the 9-2-5 introgression. This line was selected since it was observed that

5   recombinants are more efficiently obtained when long introgressions are used in the marker assisted selection. After five marker-assisted backcrosses the selfed generation of a BC5 plant that was heterozygous for the introgression was grown and the segregating population was subjected to RFLP analysis. The results were highly consistent between

10   the determinate and indeterminate backgrounds (Figure 11); the homozygous NIL, containing segment of chromosome 9, improved B by 27 % over the control with partial dominance for the wild species segment (a=0.5, d=0.25, d/a=0.5). Very similar results were obtained in another growing season (data not presented) confirming that the observed

15   effects were independent of environment in the greenhouse.

Thus, a major brix associated QTL was introgressed into a genetic background of an indeterminate greenhouse tomato (202) thus yielding plants which are high in brix and which in all other aspects are similar in phenotype to this indeterminant greenhouse tomato line. In addition, the

resultant tomato line does not display the undesirable self pruning trait inherent to determinate tomato lines specifically developed for the processing tomato industry (M82).

EXAMPLE 5

5   Thus, marker and phenotype assisted introgression studies revealed the existence of a single chromosome region which includes the high Brix QTL of green-fruited tomato fruits. The high Brix QTL (termed *Brix9-2-5*) was found to be associated with the centromeric portion of chromosome 9.

10   In order to isolate the gene or genes responsible for this phenotype further studies were conducted.

Materials and Methods:

Plant material:

The nearly isogenic lines (NILs) for the open-field trial were

15 planted in Akko, Israel (14-28 plants per NIL) in a completely randomized pattern. Agricultural practices and phenotypic measurements were described previously (Eshed and Zamir 1995, *ibid*). Glasshouse trials of the segregating recombinant families were conducted in Shekef, Israel during 1997 and 1998 in a completely randomized design.

Statistical analysis:

Statistical analysis was performed with the JMP V.3.1 software

for Macintosh. Mean brix values were compared using the "Fit Y by X"

function and "Compare with control" with an alpha level of 0.001

5   (Dunnet, 1955, J. Am. Stat. Assoc. 50, 1096-1121). The control

phenotypic values were obtained using cv. M82 for the open-field trials

(Figure 12) and with the indeterminate line 17 for the glasshouse trials

(Figure 11). The additive effect (a), dominance deviation (d) were

calculated as described above in Example 4. Mapping of *Brix9-2-5* using

10   the recombinant families was done by RFLP genotyping and a two-step

analysis. In each recombinant family the brix phenotypic value for the *L.*

pennellii homozygotes, was compared to that of line 17 and expressed as

a percentage of the control (Figure 14a). Recombinant families

containing a common marker-defined *L. pennellii* chromosome segment

15   were grouped and the mean phenotypic effects for the groups were

calculated (Figures 13 and 14a).

Nucleic acid analysis:

The different segregating populations were subjected to RFLP

analysis as previously reported (Eshed and Zamir 1996, *ibid*). A bacterial

artificial chromosome designated BAC91A4 was isolated, subcloned, sequenced and assembled by the Sequencer software package. The nucleic acid sequence of the 13 Kb insert of BAC91A4 is presented in SEQ ID NO:1. DNA of the homozygous recombinants was used as a

5 substrate for PCR, using the primers 5'-TTTGGGCTCATTCAGTCTCA-3' (SEQ ID NO:2) and 5'-AAATTGTTCGGCCTCGTT-3' (SEQ ID NO:3) in order to amplify a 1,200 bp portion of the *Lin5* gene (Figure 14b). The PCR products were cloned and sequenced using the pGEM-T easy vector by Promega. PCR was performed using PCR Supermix (Life

10 Technologies) with 35 cycles of 30 sec at 94 °C, 30 sec at 52 °C and 1 min at 68 °C, followed by 30 min at 68 °C.

Results

For fine mapping of *Brix9-2-5,* 7,000 F2 progenies of the NILs hybrids (described under Example 4) were subjected to RFLP analysis.

15 Of 145 recombinants identified between the CP44 and TG225 markers (Figure 13), 29 were further localized between the two ends of a BAC clone (BAC91A4) (Figure 13). For each of the 29 recombinant families, 48 selfed progenies were genotyped with the appropriate segregating markers and analyzed for brix. On the basis of common introgressed

segments in the 29 recombinant families, six recombination groups were generated (Figure 13). Group α1 included seven families that contained the *L. pennellii* segment north of 91H6, none of which showed a significant effect on brix. The reciprocal recombination group α2

5   contained the *L. pennellii* segment south of p14 and showed a significant increase in brix. The α groups placed the QTL south of 91H6. Using the same procedure, groups β and γ located *Brix9-2-5* between H14 and p14. To narrow the position of *Brix9-2-5*, 18-Kb spanning p14 and H14 was sequenced and used to design different primer pairs that amplified

10   polymorphic products (in size or restriction pattern) between the parental lines. These products were genetically mapped using the 29 recombinants and one of these PCR markers (F8785, Figure 13) co-segregated with the brix QTL. This 1 Kb genomic interval, represented by F8785, was sequenced in both the parental types and the

15   recombinants. Based on nucleotide polymorphisms (NPs), 13 families were shown to be recombinants within this 1-Kb fragment. The phenotypic effects for each of the 13 families were used to determine the location of *Brix9-2-5* on the NPs map (Figure 14b, SEQ ID NO:5). Recombinants 3, 13 and 6 delimited the *Brix9-2-5* to a region south of

position 2324 (Figure 14b) in a manner consistent with the mapping of the rest of the recombinant families to the north. Recombinant 2 delimited the QTL to the region north of position 2808 (Figure 14b, SEQ ID NO:5), a conclusion that is in agreement with the mapping of recombinants 5 (a member of group β1; Figure 13) and 29. This NPs mapping positioned the *Brix9-2-5* at a 484-bp fragment interval between positions 2324 and 2808 (SEQ ID NO: 4) of SEQ ID NO:5.

A GeneBank search revealed that the terminal portions of the brix QTL 484-bp interval contained regions encoding *Lycopersicon* apoplastic invertase (*Lin5* GeneBank Accession number X91389) which is expressed exclusively in flowers and fruits (Godt et al, 1997 Plant Physiol. 115, 273-282) and for which a complete nucleic acid sequence is yet to be determined.

A comparison of the genomic DNA sequenced in the present study (SEQ ID NO:1) and the cDNA sequence of *Lin5* resolved the genomic sequence of *Lin5* (SEQ ID NO:5) which includes six exons that encode the invertase protein (SEQ ID NO:6). The 484-bp interval spans a 3' portion of exon 3, intron 3 and the 5' portion of exon 4 (Figure 14b). *Lin5* is a member of a small family of genes encoding apoplastic

invertases which irreversibly cleave sucrose into glucose and fructose. In

most plant species, assimilated carbon is transported as sucrose. The

extracellular invertases maintain a gradient of carbohydrates, from the

source parts of the plant, to the developing sink tissues. In addition to

5 sucrose hydrolysis, invertase plays a central role in regulating,

amplifying, and integrating different signals that lead to source-sink

transition (Roitsch, 1999, Curr. Opin. Plant Biol. 2, 198-206). The

activity of this enzyme changes the sugar influx, and thus alters the

expression of sugar-responsive genes in a manner that is yet unclear

10 (Sturm and Tang, 1999, Trends Plant Sci. 4, 401-407).

The proposed cDNA sequence of the *Lin5* invertase from *L.

pennelli* (SEQ ID NO:7) is identical in it's 3' region to the partial *Lin5*

cDNA sequence. In addition a cDNA library from *L. pennellii* was

screened and the full length pLin5 cDNA clone was isolated and

15 sequenced. The nucleic acid sequence of this cDNA clone is identical to

SEQ ID NO: 7.

The novel invertase gene associated with the brix QTL which was

isolated as part of this study was designated pLin5. The proposed cDNA

sequence of this gene displayed a high degree of homology (identity)

(97.7 %) between the *L. pennelli* and *L. esculentum* species, while homology to other isolated invertase cDNAs was less than 76 % (Table 8).

Sequence analysis to the 13 Kb sequence (SEQ ID NO:1)
5   downstream of the pLin5 gene revealed the existence of an additional invertase gene which is referred to herein as eLin7. The genomic sequence of eLin7 (SEQ ID NO:8) was determined by BLAST analysis of the genomic sequence 3' to pLin5. Exon 1 of eLin7 is thought to be a pseudo sequence due to low sequence homology to known invertases and
10   due to the presence of two stop codons therein. The open reading frame encoding eLin7 starts from exon 3, where the homology to known genes increases dramatically (Figure 15).

Tables 5 and 6 below detail the nucleotide coordinates for the various regions in the pLin5 and eLin7 genes (numbers refer to SEQ ID
15   NO:1).

Table 5
Nucleotide coordinates of the various regions in the Lin5 Gene

Nucleotides	Description	comments
1 - 4849	Lin5 promoter	
4850 - 5048	Lin5 exon 1	4850 - start codon (#1 in the pLin5 sequence, SEQ ID NOs:5 and 7)
5049 - 6332	Lin5 intron 1	
6333 - 6341	Lin5 exon 2	Conserved in plants
6342 - 6418	Lin5 intron 2	
6419 - 7440	Lin5 exon 3	
7441 - 7619	Lin5 intron 3	Include a 30 aa ORF in L. pennelli (SEQ ID NO: 10)
7620 - 7864	Lin5 exon 4	
7865 - 8054	Lin5 intron 4	
8055 - 8154	Lin5 exon 5	
8155 - 8285	Lin5 intron 5	
8286 - 8670	Lin5 exon 6	8463 - stop codon

Table 6
Nucleotide coordinates of the various regions in the Lin7 Gene

8671 - 9981	unknown	
9982 - 10185	Lin7 pseudo exon 1	By BLAST, include two STP codons
10186 - 10549	Lin7 pseudo intron 1	
10550 - 10558	Lin7 pseudo exon 2	
10559 - 10781	Lin7 pseudo intron 2	
10782 - 11800	Lin7 exon 3	High homology to known invertases starts here and proceeds downstream
11801 - 12528	Lin7 intron 3	
12529 - 12773	Lin7 exon 4	
12774 - 12871	Lin7 intron 4	
12872 - 12968	Lin7 exon 5	
12969 - 13043	Lin7 intron 5	
13044 - 13226	Lin7 exon 6	13224 - stop codon

Using blastn (Entrez) the full sequences of the *L. pennellii* pLin5 and eLin7 cDNAs (SEQ ID NOs:7 and 9) were compared to non-redundant (nr) and expressed sequence tag (est) libraries. Homologous sequences are presented in Table 7 and the degree of homology in Table 8.

Table 7
Sequences displaying homology to the *L. pennellii Lin5* cDNA

	NID	species	PID	Blast score (gapped)
1	3608172	*L.esculentum*	3608173	5e-60
2	313128	*S.tuberosum*	313129	3e-55
3	551258	*N.tabacum*	551259	6e-47
4	170361	*L.esculentum*	170362	
5	2177080	*L.esculentum*	546937	
6	2175258	*L.esculentum*	287474	
7	TC4315	*L.esculentum*		
8	pLin5	*L.pennellii*		
9	eLin5	*L.esculentum*		
10	eLin7	*L.esculentum*		

5

10

Table 8
**Pairwise percent identity between cDNAs of the various invertases
presented in Table 7**

	3608172	313128	551258	170361	2177080	21775258	TC4315	pLin5	eLin5	eLin7
3608172		76.0%	82.9%	63.4%	63.2%	64.2%	61.9%	75.6%	75.0%	75.8%
313128	76.0%		76.4%	62.4%	62.3%	63.1%	63.6%	74.9%	73.9%	74.5 %
551258	82.9%	76.4%		61.6%	61.2%	62.1%	62.7%	73.8%	73.8%	76.5 %
170361	63.4%	62.4%	61.6%		97.7%	98.6%	68.1%	65.6%	64.4%	62.1 %
2177080	63.2%	62.3%	61.2%	97.7%		91.5%	68.0%	65.3%	64.4%	61.8 %
21775258	64.2%	63.1%	62.1%	98.6%	91.5%		68.9%	66.5%	65.2%	62.8 %
TC4315	61.9%	63.6%	62.7%	68.1%	68.0%	68.9%		67.7%	67.2%	61.9 %
pLin5	75.6%	74.9%	73.8%	65.6%	65.3%	66.5%	67.7%		97.7%	79.6 %
eLin5	75.0%	73.9%	73.8%	64.4%	64.4%	65.2%	67.2%	97.7%		79.7 %
eLin7	75.8%	74.5 %	76.5 %	62.1 %	61.8 %	62.8 %	61.9 %	79.6 %	79.7 %	

Table 9
**Pairwise percent identity between proteins translated from the cDNA
sequences of pLin 5 eLin 5 and eLin7 and database sequences**

	3608173	313129	551259	170362	546937	287474	TC4315	pLin 5	eLin 5	eLin7
3608173		77.7%	85.9%	45.0%	44.3%	44.0%	ND	72.5%	72.2%	72.9 %
313129	77.7%		79.8%	43.4%	43.1%	39.8%	ND	74.3%	73.1%	72.7 %
551259	85.9%	79.8%		44.7%	44.0%	40.0%	ND	74.8%	74.3%	76.6 %
170362	45.0%	43.4%	44.7%		99.1%	98.0%	ND	44.0%	44.2%	43.1 %
546937	44.3%	43.1%	44.0%	99.1%		97.1%	ND	43.5%	43.7%	42.4 %
287474	44.0%	39.8%	40.0%	98.0%	97.1%		ND	40.0%	40.0%	38.7 %
TC4315	ND	ND	ND	ND	ND	ND				
pLin5	72.5%	74.3%	74.8%	44.0%	43.5%	40.0%	ND		96.8%	77.4 %
eLin5	72.2%	73.1%	74.3%	44.2%	43.7%	40%%	ND	96.8%		76.7 %
eLin7	72.9 %	72.7 %	76.6 %	43.1 %	42.4 %	38.7 %	ND	77.4 %	76.7 %	

The proposed translated protein of pLin5 (SEQ ID NO:6) shows a
high degree of sequence identity to *L. Pennellii* and *L. esculentum*, while
identity to other invertase proteins (partial sequences) for both pLin5 and
eLin7 was less than 75 %. The identity between pLin5 and eLin7 was
about 77 % (Table 9).

The homology (identity + similarity) between the apoplastic
invertases (partial sequences) to the pLin5 translated protein sequence is
85-86 % (not shown), whereas for the vacoular invertases the homology
is below the detection threshold of the blast search.

Clearly the high sequence homology between the *L. pennellii* and *L. esculentum* Lin5 cDNAs and translated proteins (97.7 % and 96.8 %, respectively) suggests that differences in the genomic level, e.g. expression regulation, alternative splicing, RNA editing and the like

5 which arise from the third intron segment may be responsible for the high brix value unique to *L. pennellii*. Comparison of the *L. pennellii* and *L. esculentum* sequences revealed several differences that may be responsible for the effect of *Brix9-2-5* (Figure 14b).

(i) The *L. pennellii* third intron was longer than its

10 corresponding sequence in *L. esculentum* (201 bp vs. 179 bp) and included two 18-bp perfect direct repeats, as compared to a difference of one nucleotide between the direct repeats of *L. esculentum*. *L. pennellii* carried a 7-bp triple repeat 5' to the first direct repeat, while in *L. esculentum* both the triple repeats were deleted. These repeats may

15 regulate the expression of *Lin5* or other genes, as was recently demonstrated for a 73-bp enhancer with similar structures in rat (Hung and Penning, 1999, Mol. Endocrin. 13, 1704-1717).

(ii) A potentially important difference between the alleles of the two species relates to the downstream sequence of the first 18-bp

direct repeat. In *L. pennellii*, starting from position 2694, there is a hypothetical ORF of 30 amino acids (SEQ ID NO:10) (with no uncovered homologies in GenBank), whereas in *L. esculentum* there is a 19-bp deletion followed by stop codons. The above described structural

5   components of the 484-bp brix QTL implicates a plethora of potential biological control sequences which might be cumulatively or individually responsible for the differences in fruit sugar content. The results presented herein confirmed that *Lin5* transcripts are found exclusively in developing carpels and young fruits (Godt et al, 1997 Plant Physiol. 115,

10   273-282), however, no clear differences were detected between the *Brix9-2-5* NILs.

In addition, since the presence of a second invertase was detected downstream to the pLin5 gene, other and more complex regulatory mechanisms may be responsible for the elevated brix value in the

15   introgressed plants.

For example, the 484-bp Brix QTL region which is part of intron 3 of pLin5 or the 30 amino acid open reading frame positioned therein may function as a regulatory element (transcriptional or translational) to upregulates the expression of the eLin7 gene.

Alternatively, two distinct mRNAs resulting from alternative splicing can lead to the generation of two distinct invertase enzymes. A first invertase enzyme can be encoded by a short transcript that spans exon 3 to exon 6 of eLin7 (SEQ ID NO:9) while a second invertase

5 enzyme can be encoded by a chimeric transcript which includes exons 1 and 2 from pLin5 and exons 3-6 from eLin7 (SEQ ID NO:11).

Thus, alternative splicing of pLin 5 and pLin 7 can generate two distinct invertase enzymes (SEQ ID NOs:12 and 13 translated from SEQ ID NOs: 9 and 11, respectively) which as a result of unique N-terminal

10 regions are differentially expressed in a tissue specific pattern.

The mapping of the brix QTL was facilitated by the nearly isogenic nature of the phenotyped segregating populations where all the genetic variation for the quantitative trait was associated with the introgressed segment. The recombination hotspot created multiple

15 isogenic chimeric alleles that delimited the QTL to a defined sequence. This hotspot, which may be associated with the direct repeats in intron 3, created 13 recombinants within a 948-bp interval as compared to only 16 recombinants for the rest of the 100-Kb BAC. This observation is consistent with studies in maize, where intragenic recombination

frequencies were found to be several times greater than recombination between genes (Dooner and Martinez-Ferez, 1997, Plant Cell 9, 1633-1646 (1997).

Much of our understanding of development is based on analysis of

5   mutants which display a loss of function. However, the variation of greatest interest is often quantitatively inherited and originates from natural populations. To determine the molecular basis of such traits, it is necessary to clone the genes and devise molecular and genetic complementation approaches sensitive enough to detect minor variations

10   in gene expression pattern and function. This study highlights the potential of wild species alleles for unraveling novel variations which can be potentially useful to agricultural production.

Although the invention has been described in conjunction with

15   specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended

claims. All publications cited herein are incorporated by reference in their entirety.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i)   APPLICANT: DANI ZAMIR *ET AL.*

    (ii)   TITLE OF INVENTION: POLYNUCLEOTIDES ENCODING
                                        POLYPEPTIDES HAVING INVERTASE
                                        ACTIVITY AND USE OF SAME

    (iii)   NUMBER OF SEQUENCES: 16

    (iv)   CORRESPONDENCE ADDRESS:

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         (D)   STATE: Virginia

         (E)   COUNTRY: United States of America

         (F)   ZIP: 22202

    (v)   COMPUTER READABLE FORM:

         (A)   MEDIUM TYPE: 1.44 megabyte, 3.5" microdisk

         (B)   COMPUTER: Twinhead* Slimnote-890TX

         (C)   OPERATING SYSTEM: MS DOS version 6.2,
                                          Windows version 3.11

         (D)   SOFTWARE: Word for Windows version 2.0
                                          converted to an ASCI file

    (vi)   CURRENT APPLICATION DATA:

         (A)   APPLICATION NUMBER:

         (B)   FILING DATE:

         (C)   CLASSIFICATION:

    (vii)   PRIOR APPLICATION DATA:

         (A)   APPLICATION NUMBER:

         (B)   FILING DATE:

    (viii) ATTORNEY/AGENT INFORMATION:

         (A)   NAME: Friedmam, Mark M.

         (B)   REGISTRATION NUMBER: 33,883

         (C)   REFERENCE/DOCKET NUMBER: 325/78

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         (A)   TELEPHONE: 972-3-5625553

         (B)   TELEFAX: 972-3-5625554

         (C)   TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

    (i)   SEQUENCE CHARACTERISTICS:

         (A)   LENGTH: 13,226

         (B)   TYPE: nucleic acid

         (C)   STRANDEDNESS: double

         (D)   TOPOLOGY: linear

    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCAAAAACAA TCGCAAATGA ATAAAGATGC CCAAAATAGA GTAACCTAAC 9150
GAAGTCGATA CTTCCATTCA TAATCAAATC TGTTCAAAAA CACTTGATGG 9200
TTTATTTTTA ACTTTAAGAG ATGTATCATA TCGTCTCTTA TTATTCTTTT 9250
AGGGCTATTC GCCGTAGGAA TAAAATTTAT ATGATCAAAT TTCACGTTAT 9300
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ATAAATAATG TGAAGAAAAA ACTTATACTT TTCAAGGTAA CAAGAAATCA 9350
TGTTTTTTTT ACGCCTTCGT GGAGACTACT TCCTCGTAAC AAAAAATTAA 9400
CATTTTAAGT GGCGACTCTA AAAACTCGTG GCCAGTATAT TAGTCGCCAT 9450
TAAACATTAT TTTTAATCAT GAGTTCTTTT CTTTTTTAAT CTTTTTTTAA 9500
GGTCAAATTT ACCACTTTAT CTTATTTATT TAAATTGAAA AATCCCAAAT 9550
TTTGCATTAT TTTTTTGAAT TCCTTTTTTT TTTACACACT CAAAAAGTCA 9600
AAACATTAAA AAAACGAAAT AGCAAATTAA ATGGCAAAAG ACTTGTTCTA 9650
ACAAAAAAAA AATAGTAAAA CAGACTCATA AAAGGTAACA ATAACCAACA 9700
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ATCCAATCCA TTTATTTATT TTTTTAAAAA AAACCTAAAT TAACTCTCCA 9800
TCTTTCAATC AAAAACAAAC TCTACCCATT TTTTTCACTA TAAATACTCT 9850
TCATAATTTT CATTTGTTCT TCATTCCCAT GTTTCTTTTC TCCTTATCCA 9900
AAAAAAAAAA AATTAAAAAA AATTATTTAG ATTAAATATC ACTATCTGTC 9950
AAAGCCCAAT CATTAAAATA AAATAAAAAT TATGGATTAT TCATCTAATA 10000
AAAGTTCTCG TTGGGCTTTG CCAGTTATCT TATTTGGCTT TTTTGGGAAT 10050
TTTATTATCC AATAATGTTG TTTTGGCTCC TCATAAAGTT TTTATTCACT 10100
TGCAATCCCA AAATGCTGTA AATGGTCATA CTGTTCATCG AACGGGTTAT 10150
CATTTTCAGC CCGAAAAACA TTGGATCAAG GGTATGTAAT CCCTTTTTTT 10200
TCGTCTTTTT TTTTAATATA TATATAATAA TAAACGACCA TGTTGTGTTT 10250
AGTCTAGATT TAATACTAGT GATTTTTTGG ACGCTAACCA AATAATGGGT 10300
ACTCACCATT TGTCAATAGA TACATTGACA TGTATTAGTA TGATTTTCGT 10350
CTTTTTTCGT TGTTTCTAAT ATTATTTAAT CTTCACTAAT TTTTTTTATT 10400
TTTCTTTGAA TGATGTCTCT TGGTCAAAAC ATACAATAGA TCCCAATGGT 10450
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ATGCACCAGT ATCCTCTGTT ATACTTTAGA GACATTTTTG CCGTCAAAAA 10600
ACTAGAACAC ATATATCCTT TATTTATCCC GATATCGAAT CGATTGTACC 10650
ACGAGTGAAG GGTATAGCTC TAGTTTTTGG ACGGTAGGGC ACCTAAAGTA 10700
TGACGAAGAA TATCTGCAAA CCATTTACAA TAGTTTTGGA TATATTTGTG 10750
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AGTGTATCAT CTATTCTACC AGTACAACCC AAATGGTTCA GTATGGGGTA 10850
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TTAGAACCTG CAATTTACCC ATCAAAGCCA TTTGATCAAT TCGGTACCTG 10950
GTCTGGATCA GCAACCATCC TACCTGGTAA CAAGCCAGTC ATCTTGTACA 11000
CCGGAATCAT AGATGCCAAC CAAACCCAAG TCCAAAACTA CGCAATCCCA 11050
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AGATGAACAA TGGGGAGAAG ATTGAAGTTA CAGGAATCAC ACCAGCACAG 11800
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GATTTTTCAC GTGAAATGTT TAAAATCACG AGATTAAAGA GCATTTTGGT 12000
TACATTTGAC ATAACTGAAA TTTAGAAACA CAAGATTAAA GGACATTTTG 12050
GTACATTTGA CATAACTTGA ATTTAAAACC ACATAATTAA AGGGCATTTT 12100
GGTACATTTG AATTAGAACA TTTTGATACA TTTGACATAA CATGAATTTA 12150
GAACCACAAG ATTAAAAAAT CTTCTTTCTT TTTTCTTAAA TTTCGTTCCA 12200
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TAACAACATT TGACCGGACG GAGAGTTTTA AGAAATAAAT AAAACACTTT 12300
GAGATGTGTA CCAAATTGCT CTCCAAAAAT ACTCACTTTT CTCTCTCCTC 12350
ATAAATGTAT TTGAGTACTA TTTTTAAAAT TAAGCGAGTC CAACAAGAAT 12400
AAAATAGAAA CTGTACTTTT AAATATTTAC CATATAAAAA AATGTGATTT 12450
TTTTTTTTTG AAAACTGATC AAAAAGAAAA TGATATCACT CGACGATGAA 12500
AGTGTTTAAT AATGAAAAAA CATGACAGGC TGATGTTGAA GTGACATTCT 12550
CATTTGCAAG TTTGGATAAG GCAGAGTCAT TTGATCCTAA ATGGAATGAT 12600
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CTCTTGTGTT CTGACGCTAA AAGGTACTAC TTATTGAATT TTTAACTTGT 12800
TGGTAACGTT TTCGACGGTA TAATATCGAG AAGTTGAGAA ATTGACAAAT 12850
CTTTTGTTTT ATGTCTGATA GGTCAACTCT TAAGTTCAAT GAAACAATGT 12900
ACAAAGCTTC ATTTGCTGGA TTTGTTGATG TTGATTGGC TGACAAGAAA 12950
TTGTCACTCA GAAGCTTGGT AACTTCTCTT TCTATCGTTA ATCAAAAATC 13000
TAAACGAACA TTTGAATCTA AACTATTGAA ATTCTTTTTG TAGATTGATA 13050
ATTCAGTTAT AGAAACTTTT GGTGCTGGTG GAAAGACATG TATAACATCG 13100
AGGGTTTATC CAACATTGGC AATTAACGAC GAGGCACATT TATTCGCGTT 13150
TAACAACGGA ACGGAGCCAA TCACAATTGA GAGTTTGGAT GCATGGAGTA 13200
TGGGCAAAGC TAAGATACAA TATTGA                          13226
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(2)   INFORMATION FOR SEQ ID NO:2:
    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 20
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear
    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:2:

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TTTGGGCTCA TTCAGTCTCA 20
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(2)   INFORMATION FOR SEQ ID NO:3:
    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 18
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear
    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AAATTGTTCG GCCTCGTT 18
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(2)   INFORMATION FOR SEQ ID NO:4:
    (i)   SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 484
        (B)   TYPE: nucleic acid
        (C)   STRANDEDNESS: double
        (D)   TOPOLOGY: linear
    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:4:

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AAATTCATAT AATTTTGAAT TGAAAGGAAA AAGAGTCAAA TTCAAAATTA 50
CTATCGAAAT ACTTCGATTT TGTGTCTCGT GCTAAGGGAA ATGGTGAACG 100
AATGTCAGAA GAGGGATGGG GTGGGATGTT CTGGGAAGAA GAAGAAGAAA 150
AGGGGAACGA AATATTTTTT TTTAATTATT TTTTTTAGTG AAAACATAAT 200
TTTTGTTTAT TTTATTTTTT TAATTTATGT TTTAAGTGTG TTTATGGGCC 250
CAATGCCACA TGTCTCTTTT TTATTAGCTA TTATTTCACG TTAGCAGCGA 300
GTGTAGGACA TTCTCTCTTT ACTTTTAATT AATTGTCAAA ATATATATAT 350
ATCTCTTTAA AATACACTAT TAAATAATAC ATCGATAAAA TATCTTTTCC 400
AAAATTTGAT TATTATTACA ATAATTTCGA TCAAAATTCA TGAGGTGTTT 450
TTTCTTTTTT TATATACAAT TCACTAATAT TTGG                 484
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(2)   INFORMATION FOR SEQ ID NO:5:

    (i)   SEQUENCE CHARACTERISTICS:

        (A)   LENGTH: 3616

        (B)   TYPE: nucleic acid

        (C)   STRANDEDNESS: double

        (D)   TOPOLOGY: linear

    (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATTTTGCTTA TTTATAATTT TATCAAACAT TAATAGGGCA TTTGCTTCTC 100
ATAATATTTT TTTGGACTTG CAATCTTCAA GTGCTATTAG TGTCAAGAAT 150
GTTCATAGAA CTCGTTTTCA TTTTCAACCT CCTAAACATT GGATTAATGG 200
TATGTTCATT TTTTTTTTAT TTTATATAAC ATGCGATAAA TTTAACGTTA 250
GCAATGTGGT TTGTTATTTA AATTCGAATT TGATTATATG ACTTTGCTTA 300
TATAAATATA CATAGTAATA AAAGTTTGTG TATAAATGCA TGTCATATAC 350
ATTTATTGAC TTGGTATATA TATCAGTACG ATTAAATTAA TTGATGGTGC 400
AATTAATATT GCATTATTTA GGTGATAAAA CTACAGAAAT TAACGAAAAT 450
ATTTTTTTTA TATAGAGAAG TTCAAATGTT GAGGGTTCTT TTATGGTTAC 500
ATTGGTTTAA AATGTTTTTT GTTAACTATC TTTATAGCTA CATATATATA 550
AGAGTGATCA TTCTTTATAT TTCAAAATTA TATCTACATA CACACATATA 600
CATCATTATG TGGTTCATTT ATGGTAGTTT TCAGTATTCG ATATTTATTT 650
TTAAGTTTAA TTTATTTAAA TCTGCGTTAA AATATCTCAC TTTGAAAGAT 700
AGAATCACTC CTGACCAACT ATGAGTAACT CGATTCTCAA AATTTAAATT 750
CGGAATTAGA TTAATTATCA TGGCAAGAGA ACTACCACGT TTTGGATAAG 800
AATGTGCAAA AGAGAGAAAG AAACATGAAA TATATAAAAA CCTAAGATTT 850
TGGCCATGGA AAGTTAGGTG CGAATTAATT TGTTGAAGGC ACCCTTTATT 900
ATTATTATTA TAATTATTAT TATTATTAAT GAAATATAGT GACATTTCAT 950
ACTCATATAT TGTGTGCATT TAATTAATAT ATGTAGGTCT TATGTTAATT 1000
TAAACTTACC AAACATATTG TCTCTTATAA AGTTGACTCC CCCCCTCAAC 1050
CGCCAACCCC ACCCCCACCC CCACCCCACC CAAAAAAAAT ACCTCATCAA 1100
TTTCGGTTTT TATATGACTC AATTTTCTTG TTTAATTTGT TATCTACAGA 1150
ACGGACTACT TTCTATATCA TTCTACATAA TATGTATATT TTTTATAATC 1200
CAATAAATCT CATGACACGT TTTCAGATCA TAATTTTGCA AACACCTTTT 1250
TCTTTATTTT TTAATTAGGT ATATCACATA AATTAAAAGG ATTCATTAAT 1300
TTTCGCAGAG AAAACTAATT AGTTTCTGTG TTTTTCACCT TTCATTTATT 1350
AATTACTACA TAATTTTTAA TCAATAATTG ATGAAAGACT ATGTAATGTA 1400
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CATTTTATTT TTCAAACAGC ACCAATGTAT TATAATGGAG TGTATCATTT 1600
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CTCATTCAGT CTCAAAAGAC TTGATAAATT GGATCCATTT AGAACCTGCA 1700
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GATCCATTTC TTCGTAAATG GATCAAACCT AACAACAACC CGTTGATCGT 1900
CCCTGATAAC AGTATCAATA GAACTGAGTT TCGCGATCCA ACTACAGCTT 1950
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CATAGAGGGA TGGCATTGTT GTATAGAAGT AGAGATTTTA TGAAATGGAT 2050
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GTCCTGATTT TTTCCCTGTA TTATTTAATA GTACCAATGG TTTAGATGTA 2150
TCGTATCGCG GAAAAAATGT TAAATATGTC CTCAAGAATA GTCTTGATGT 2200
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TCAAAACTTT CAAGATTCGA TTAATTTGAA GAGTAACGAT TTGTGTTTGA 3400
CTAATCAATT TGTATCATAT GCATATTTTT TTTTAGATTG ATAACTCAGT 3450
AGTGGAAAGT TTCGGTGCTG GTGGCAAAAC ATGCATAACA TCAAGGGTGT 3500
ATCCAACTTT AGCGATTTAT GATAATGCAC ATTTATTTGT TTTTAACAAT 3550
GGCTCTGAGA CAATCACAAT TGAGACTCTG AATGCTTGGA GCATGGATGC 3600
ATGTAAGATG AACTAA                                     3616
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(2)   INFORMATION FOR SEQ ID NO:6:
         (i)   SEQUENCE CHARACTERISTICS:
                 (A)   LENGTH: 584
                 (B)   TYPE: amino acid
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear
         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Tyr Leu Phe Cys Leu Phe Ile Ile Leu Ser Asn Ile Asn Arg Ala
                20                  25                  30
Phe Ala Ser His Asn Ile Phe Leu Asp Leu Gln Ser Ser Ser Ala
                35                  40                  45
Ile Ser Val Lys Asn Val His Arg Thr Arg Phe His Phe Gln Pro
                50                  55                  60
Pro Lys His Trp Ile Asn Asp Pro Asn Ala Pro Met Tyr Tyr Asn
                65                  70                  75
Gly Val Tyr His Leu Phe Tyr Gln Tyr Asn Pro Lys Gly Ser Val
                80                  85                  90
Trp Gly Asn Ile Ile Trp Ala His Ser Val Ser Lys Asp Leu Ile
                95                  100                 105
Asn Trp Ile His Leu Glu Pro Ala Ile Tyr Pro Ser Lys Lys Phe
                110                 115                 120
Asp Lys Tyr Gly Thr Trp Ser Gly Ser Ser Thr Ile Leu Pro Asn
                125                 130                 135
Asn Lys Pro Val Ile Ile Tyr Thr Gly Val Val Asp Ser Tyr Asn
                140                 145                 150
Asn Gln Val Gln Asn Tyr Ala Ile Pro Ala Asn Leu Ser Asp Pro
                155                 160                 165
Phe Leu Arg Lys Trp Ile Lys Pro Asn Asn Asn Pro Leu Ile Val
                170                 175                 180
Pro Asp Asn Ser Ile Asn Arg Thr Glu Phe Arg Asp Pro Thr Thr
                185                 190                 195
Ala Trp Met Gly Gln Asp Gly Leu Trp Arg Ile Leu Ile Ala Ser
                200                 205                 210
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Met Arg Lys His Arg Gly Met Ala Leu Leu Tyr Arg Ser Arg Asp
              215               220               225
Phe Met Lys Trp Ile Lys Ala Gln His Pro Leu His Ser Ser Thr
              230               235               240
Asn Thr Gly Asn Trp Glu Cys Pro Asp Phe Phe Pro Val Leu Phe
              245               250               255
Asn Ser Thr Asn Gly Leu Asp Val Ser Tyr Arg Gly Lys Asn Val
              260               265               270
Lys Tyr Val Leu Lys Asn Ser Leu Asp Val Ala Arg Phe Asp Tyr
              275               280               285
Tyr Thr Ile Gly Met Tyr His Thr Lys Ile Asp Arg Tyr Ile Pro
              290               295               300
Asn Asn Asn Ser Ile Asp Gly Trp Lys Gly Leu Arg Ile Asp Tyr
              305               310               315
Gly Asn Phe Tyr Ala Ser Lys Thr Phe Tyr Asp Pro Ser Arg Asn
              320               325               330
Arg Arg Val Ile Trp Gly Trp Ser Asn Glu Ser Asp Val Leu Pro
              335               340               345
Asp Asp Glu Ile Lys Lys Gly Trp Ala Gly Ile Gln Gly Ile Pro
              350               355               360
Arg Gln Val Trp Leu Asn Leu Ser Gly Lys Gln Leu Leu Gln Trp
              365               370               375
Pro Ile Glu Glu Leu Glu Thr Leu Arg Lys Gln Lys Val Gln Leu
              380               385               390
Asn Asn Lys Lys Leu Ser Lys Gly Glu Met Phe Glu Val Lys Gly
              395               400               405
Ile Ser Ala Ser Gln Ala Asp Val Glu Val Leu Phe Ser Phe Ser
              410               415               420
Ser Leu Asn Glu Ala Glu Gln Phe Asp Pro Arg Trp Ala Asp Leu
              425               430               435
Tyr Ala Gln Asp Val Cys Ala Ile Lys Gly Ser Thr Ile Gln Gly
              440               445               450
Gly Leu Gly Pro Phe Gly Leu Val Thr Leu Ala Ser Lys Asn Leu
              455               460               465
Glu Glu Tyr Thr Pro Val Phe Phe Arg Val Phe Lys Ala Gln Lys
              470               475               480
Ser Tyr Lys Ile Leu Met Cys Ser Asp Ala Arg Arg Ser Ser Met
              485               490               495
Arg Gln Asn Glu Ala Met Tyr Lys Pro Ser Phe Ala Gly Tyr Val
              500               505               510
Asp Val Asp Leu Glu Asp Met Lys Lys Leu Ser Leu Arg Ser Leu
              515               520               525
Ile Asp Asn Ser Val Val Glu Ser Phe Gly Ala Gly Gly Lys Thr
              530               535               540
Cys Ile Thr Ser Arg Val Tyr Pro Thr Leu Ala Ile Tyr Asp Asn
              545               550               555
Ala His Leu Phe Val Phe Asn Asn Gly Ser Glu Thr Ile Thr Ile
              560               565               570
Glu Thr Leu Asn Ala Trp Ser Met Asp Ala Cys Lys Met Asn
              575               580
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(2)   INFORMATION FOR SEQ ID NO:7:
         (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 1960
                  (B)   TYPE: nucleic acid
                  (C)   STRANDEDNESS: double
                  (D)   TOPOLOGY: linear
         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATGGAATTAT TTATGAAAAA CTCTTCTCTT TGGGGTTTAA AATTTTATTT 50
ATTTTGCTTA TTTATAATTT TATCAAACAT TAATAGGGCA TTTGCTTCTC 100
ATAATATTTT TTTGGACTTG CAATCTTCAA GTGCTATTAG TGTCAAGAAT 150
GTTCATAGAA CTCGTTTTCA TTTTCAACCT CCTAAACATT GGATTAATGA 200
CCCTAATGCA CCAATGTATT ATAATGGAGT GTATCATTTA TTCTATCAAT 250
ACAATCCAAA AGGATCAGTA TGGGGCAATA TTATTTGGGC TCATTCAGTC 300
TCAAAAGACT TGATAAATTG GATCCATTTA GAACCTGCAA TTTATCCATC 350
CAAAAAATTT GACAAGTATG GTACTTGGTC TGGATCATCA ACTATTTTAC 400
CTAATAACAA ACCTGTTATC ATATACACCG GAGTAGTAGA TTCGTATAAT 450
AATCAAGTCC AGAACTACGC CATCCCGGCT AACCTATCTG ATCCATTTCT 500
TCGTAAATGG ATCAAACCTA ACAACAACCC GTTGATCGTC CCTGATAACA 550
GTATCAATAG AACTGAGTTT CGCGATCCAA CTACAGCTTG GATGGGCCAA 600
GATGGGCTTT GGAGGATTTT AATAGCAAGT ATGAGAAAAC ATAGAGGGAT 650
GGCATTGTTG TATAGAAGTA GAGATTTTAT GAAATGGATC AAAGCCCAAC 700
ATCCACTTCA TTCATCTACT AATACTGGAA ATTGGGAGTG TCCTGATTTT 750
TTCCCTGTAT TATTTAATAG TACCAATGGT TTAGATGTAT CGTATCGCGG 800
AAAAAATGTT AAATATGTCC TCAAGAATAG TCTTGATGTT GCTAGGTTTG 850
ATTATTACAC TATTGGCATG TATCACACCA AAATAGATAG GTATATTCCG 900
AATAACAATT CAATTGATGG TTGGAAGGGA TTGAGAATCG ACTATGGTAA 950
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TAGTGGTAAA CAATTACTTC AATGGCCTAT TGAAGAATTA GAAACCCTAA 1150
GGAAGCAAAA GGTCCAATTG AACAACAAGA AGTTGAGCAA GGGAGAAATG 1200
TTTGAAGTTA AAGGGATCTC AGCATCACAG GCTGATGTTG AAGTGCTGTT 1250
CTCATTTTCA AGTTTGAACG AGGCCGAACA ATTTGATCCT AGATGGGCTG 1300
ACCTATATGC CCAAGACGTT TGTGCCATTA AGGGTTCGAC TATCCAAGGT 1350
GGGCTTGGAC CATTTGGGCT TGTGACATTA GCTTCTAAAA ACTTAGAAGA 1400
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TTCTCATGTG CTCAGATGCT AGAAGATCTT CCATGAGACA AAATGAAGCA 1500
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GAAGAAGTTA TCTCTTAGGA GTTTGATTGA TAACTCAGTA GTGGAAAGTT 1600
TCGGTGCTGG TGGCAAAACA TGCATAACAT CAAGGGTGTA TCCAACTTTA 1650
GCGATTTATG ATAATGCACA TTTATTTGTT TTTAACAATG GCTCTGAGAC 1700
AATCACAATT GAGACTCTGA ATGCTTGGAG CATGGATGCA TGTAAGATGA 1750
ACTAAATATT TTCAAAAAAA TTGGAATTAT GTCTACAATT ATATATGTCT 1800
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TCCTCTATAA TTGTCTCTAA TTTATTTTGG TGAATTTAGA AGGCAAAGTG 1900
TGTGTATGGA TTTTTCTAGT ACCATATATA TATATATTAA GTAAGAAATT 1950
TGTTAGCTTT                                           1960
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(2)     INFORMATION FOR SEQ ID NO:8:
        (i)     SEQUENCE CHARACTERISTICS:
                (A)     LENGTH:         3245
                (B)     TYPE:           nucleic acid
                (C)     STRANDEDNESS:   double
                (D)     TOPOLOGY:       linear
        (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:8:
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ATGGATTATT CATCTAATAA AAGTTCTCGT TGGGCTTTGC CAGTTATCTT 50
ATTTGGCTTT TTTGGGAATT TTATTATCCA ATAATGTTGT TTTGGCTCCT 100
CATAAAGTT TTTATTCACTT GCAATCCCAA AATGCTGTAA ATGGTCATAC 150
TGTTCATCG AACGGGTTATC ATTTTCAGCC CGAAAAACAT TGGATCAAGG 200
GTATGTAAT CCCTTTTTTTT CGTCTTTTTT TTTAATATAT ATATAATAAT 250
AAACGACCA TGTTGTGTTTA GTCTAGATTT AATACTAGTG ATTTTTTGGA 300
CGCTAACCA AATAATGGGTA CTCACCATTT GTCAATAGAT ACATTGACAT 350
GTATTAGTA TGATTTTCGTC TTTTTTCGTT GTTTCTAATA TTATTTAATC 400
TTCACTAAT TTTTTTTATTT TTCTTTGAAT GATGTCTCTT GGTCAAAACA 450
TACAATAGA TCCCAATGGTA AGTTAACTAT ATTTTTGTAT ATTTTTTAAA 500
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TTTATTTTA  TTCTTATTATA  TAATATAGGG  AAAAAAGGAT  AAATATATCC  550
CCGAACTAT  TATAAATAGTA  TGCACCAGTA  TCCTCTGTTA  TACTTTAGAG  600
ACATTTTTG  CCGTCAAAAAA  CTAGAACACA  TATATCCTTT  ATTTATCCCG  650
ATATCGAAT  CGATTGTACCA  CGAGTGAAGG  GTATAGCTCT  AGTTTTTGGA  700
CGGTAGGGC  ACCTAAAGTAT  GACGAAGAAT  ATCTGCAAAC  CATTTACAAT  750
AGTTTTGGA  TATATTTGTGA  ACTAATGATG  TTTGAATTCT  TTTTTCATAG  800
CACCAATGT  ATTTCAATGGA  GTGTATCATC  TATTCTACCA  GTACAACCCA  850
AATGGTTCA  GTATGGGGTAA  CATTGTTTGG  GCTCATTCCG  TTTCAAAAGA  900
CTTGATCAA  TTGGATCAATT  TAGAACCTGC  AATTTACCCA  TCAAAGCCAT  950
TTGATCAAT  TCGGTACCTGG  TCTGGATCAG  CAACCATCCT  ACCTGGTAAC  1000
AAGCCAGTC  ATCTTGTACAC  CGGAATCATA  GATGCCAACC  AAACCCAAGT  1050
CCAAAACTA  CGCAATCCCAG  CTAACTTATC  CGATCCATAT  CTCCGCGAAT  1100
GGATCAAGC  CAGACAACAAC  CCATTAATTA  TAGCCGATGA  AAGTATCAAC  1150
AAGACCAAG  TTTCGTGACCC  AACAACAGCA  TGGATGGGTA  AAGACGGGCA  1200
TTGGAGAAT  CGTCATGGGAA  GTTTGAGGAA  ACACAGCAGG  GGCTTAGCTA  1250
TAATGTATA  GGAGCAAAGAC  TTTATGAAAT  GGGTCAAGGC  TAAACACCCA  1300
CTTCACTCA  ACTAACGGCAC  TGGAAACTGG  GAATGCCCTG  ATTTTTACCC  1350
AGTTTCATC  GAAAGGTACTG  ATGGGTTGGA  TCAATACGGT  GAGGAACACA  1400
AGTACGTGC  TGAAGAACAGT  ATGGATCTTA  CTCGATTTGA  GTATTATACA  1450
CTTGGAAAA  TACGATACGAA  AAAAGATAGG  TACGTTCCAG  ATCCAGATTC  1500
TGTCGATAG  TTTGAAGGGAT  TGAGACTCGA  TTACGGTAAC  TTCTACGCAT  1550
CGAAGTCAT  TCTACGATCCA  AGCAAAAATC  GAAGGGTTAT  CTGGGGTTGG  1600
TCTAATGAA  TCAGATATATT  CCCAGAGGAT  GATAATGCGA  AGGGATGGGC  1650
TGGGATTCA  ATTGATTCCTC  GTAAAGTATG  GCTTGATCCA  AGTGGTAAGC  1700
AGTTGGTTC  AATGGCCTGTG  GAGGAACTAG  AAACCCTAAG  AACTCAAAAG  1750
GTTCAATTG  AGCAACAAGAA  GATGAACAAT  GGGGAGAAGA  TTGAAGTTAC  1800
AGGAATCAC  ACCAGCACAGG  TATATATATA  GACTTTTTTA  TTTTTAATTT  1850
ATTATTATT  ATTATTATTAC  TCTCTCCGTT  TTCAAAAAAA  AAATATCCCT  1900
ATTTTCTTT  TATAGTCTCTT  TAATTTAAAA  AGAATGATCT  ATTTTCTTTT  1950
TGGATAACC  TTTTAACTTTG  ATTTTTCACG  TGAAATGTTT  AAAATCACGA  2000
GATTAAAGA  GCATTTTGGTT  ACATTTGACA  TAACTGAAAT  TTAGAAACAC  2050
AAGATTAAA  GGACATTTTGG  TACATTTGAC  ATAACTTGAA  TTTAAAACCA  2100
CATAATTAA  AGGGCATTTTG  GTACATTTGA  ATTAGAACAT  TTTGATACAT  2150
TTGACATAA  CATGAATTTAG  AACCACAAGA  TTAAAAAATC  TTCTTTCTTT  2200
TTTCTTAAA  TTTCGTTCCAA  GTCAAATTAG  GTCATTCTTT  TTTAATTACT  2250
CCCTCCGTC  TAATTTTATGT  AACAACATTT  GACCGGACGG  AGAGTTTTAA  2300
GAAATAAAT  AAAACACTTTG  AGATGTGTAC  CAAATTGCTC  TCCAAAAATA  2350
CTCACTTTT  CTCTCTCCTCA  TAAATGTATT  TGAGTACTAT  TTTTAAAATT  2400
AAGCGAGTC  CAACAAGAATA  AAATAGAAAC  TGTACTTTTA  AATATTTACC  2450
ATATAAAAA  AATGTGATTTT  TTTTTTTTGA  AAACTGATCA  AAAAGAAAAT  2500
GATATCACT  CGACGATGAAA  GTGTTTAATA  ATGAAAAAAC  ATGACAGGCT  2550
GATGTTGAA  GTGACATTCTC  ATTTGCAAGT  TTGGATAAGG  CAGAGTCATT  2600
TGATCCTAA  ATGGAATGATA  TGTATGCACA  AGATGTTTGT  GGACTCAAGG  2650
GTGCAGATG  TTCAAGGTGGG  CTTGGGCCAT  TTGGTCTTGC  TACATTAGCT  2700
ACTGAAAAC  TTGGAAGAAAA  CACACCGGTT  TTCTTCCGAG  TTTTCAAAGC  2750
ACAGCAAAA  CTACAAGGTTC  TCTTGTGTTC  TGACGCTAAA  AGGTACTACT  2800
TATTGAATT  TTTAACTTGTT  GGTAACGTTT  TCGACGGTAT  AATATCGAGA  2850
AGTTGAGAA  ATTGACAAATC  TTTTGTTTTA  TGTCTGATAG  GTCAACTCTT  2900
AAGTTCAAT  GAAACAATGTA  CAAAGCTTCA  TTTGCTGGAT  TTGTTGATGT  2950
TGATTTGGC  TGACAAGAAAT  TGTCACTCAG  AAGCTTGGTA  ACTTCTCTTT  3000
CTATCGTTA  ATCAAAAATCT  AAACGAACAT  TTGAATCTAA  ACTATTGAAA  3050
TTCTTTTTG  TAGATTGATAA  TTCAGTTATA  GAAACTTTTG  GTGCTGGTGG  3100
AAAGACATG  TATAACATCGA  GGGTTTATCC  AACATTGGCA  ATTAACGACG  3150
AGGCACATT  TATTCGCGTTT  AACAACGGAA  CGGAGCCAAT  CACAATTGAG  3200
AGTTTGGAT  GCATGGAGTAT  GGGCAAAGCT  AAGATACAAT  ATTGA       3245
```

INFORMATION FOR SEQ ID NO:9:
        (i)   SEQUENCE CHARACTERISTICS:

```
                    (A)    LENGTH:           1539
                    (B)    TYPE:             nucleic acid
                    (C)    STRANDEDNESS:     double
                    (D)    TOPOLOGY:         linear
           (xi)    SEQUENCE DESCRIPTION:  SEQ ID NO:9:
ATGTATTTCA ATGGAGTGTA TCATCTATTC TACCAGTACA ACCCAAATGG 50
TTCAGTATGG GGTAACATTG TTTGGGCTCA TTCCGTTTCA AAAGACTTGA 100
TCAATTGGAT CAATTTAGAA CCTGCAATTT ACCCATCAAA GCCATTTGAT 150
CAATTCGGTA CCTGGTCTGG ATCAGCAACC ATCCTACCTG GTAACAAGCC 200
AGTCATCTTG TACACCGGAA TCATAGATGC CAACCAAACC CAAGTCCAAA 250
ACTACGCAAT CCCAGCTAAC TTATCCGATC CATATCTCCG CGAATGGATC 300
AAGCCAGACA ACAACCCATT AATTATAGCC GATGAAAGTA TCAACAAGAC 350
CAAGTTTCGT GACCCAACAA CAGCATGGAT GGGTAAAGAC GGGCATTGGA 400
GAATCGTCAT GGGAAGTTTG AGGAAACACA GCAGGGGCTT AGCTATAATG 450
TATAGGAGCA AAGACTTTAT GAAATGGGTC AAGGCTAAAC ACCCACTTCA 500
CTCAACTAAC GGCACTGGAA ACTGGGAATG CCCTGATTTT TACCCAGTTT 550
CATCGAAAGG TACTGATGGG TTGGATCAAT ACGGTGAGGA ACACAAGTAC 600
GTGCTGAAGA ACAGTATGGA TCTTACTCGA TTTGAGTATT ATACACTTGG 650
AAAATACGAT ACGAAAAAAG ATAGGTACGT TCCAGATCCA GATTCTGTCG 700
ATAGTTTGAA GGGATTGAGA CTCGATTACG GTAACTTCTA CGCATCGAAG 750
TCATTCTACG ATCCAAGCAA AAATCGAAGG GTTATCTGGG GTTGGTCTAA 800
TGAATCAGAT ATATTCCCAG AGGATGATAA TGCGAAGGGA TGGGCTGGGA 850
TTCAATTGAT TCCTCGTAAA GTATGGCTTG ATCCAAGTGG TAAGCAGTTG 900
GTTCAATGGC CTGTGGAGGA ACTAGAAACC CTAAGAACTC AAAAGGTTCA 950
ATTGAGCAAC AAGAAGATGA ACAATGGGGA GAAGATTGAA GTTACAGGAA 1000
TCACACCAGC ACAGGCTGAT GTTGAAGTGA CATTCTCATT TGCAAGTTTG 1050
GATAAGGCAG AGTCATTTGA TCCTAAATGG AATGATATGT ATGCACAAGA 1100
TGTTTGTGGA CTCAAGGGTG CAGATGTTCA AGGTGGGCTT GGGCCATTTG 1150
GTCTTGCTAC ATTAGCTACT GAAAACTTGG AAGAAAACAC ACCGGTTTTC 1200
TTCCGAGTTT TCAAAGCACA GCAAAACTAC AAGGTTCTCT TGTGTTCTGA 1250
CGCTAAAAGG TCAACTCTTA AGTTCAATGA AACAATGTAC AAAGCTTCAT 1300
TTGCTGGATT TGTTGATGTT GATTTGGCTG ACAAGAAATT GTCACTCAGA 1350
AGCTTGATTG ATAATTCAGT TATAGAAACT TTTGGTGCTG GTGGAAAGAC 1400
ATGTATAACA TCGAGGGTTT ATCCAACATT GGCAATTAAC GACGAGGCAC 1450
ATTTATTCGC GTTTAACAAC GGAACGGAGC CAATCACAAT TGAGAGTTTG 1500
GATGCATGGA GTATGGGCAA AGCTAAGATA CAATATTGA           1539
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Met Ile Asp Arg Tyr Ile Tyr Ser Arg Leu Thr Ile His Leu Leu
                5                10                      15
Tyr Ile Ser Ile Ile Ser Ile Ala Leu Ser His Leu Ala Phe Leu
                20               25                      30
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ATGGAATTAT TTATGAAAAA CTCTTCTCTT TGGGGTTTAA AATTTTATTT 50
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ATTTTGCTTA TTTATAATTT TATCAAACAT TAATAGGGCA TTTGCTTCTC 100
ATAATATTTT TTTGGACTTG CAATCTTCAA GTGCTATTAG TGTCAAGAAT 150
GTTCATAGAA CTCGTTTTCA TTTTCAACCT CCTAAACATT GGATTAATGA 200
CCCTAATGCA CCAATGTATT TCAATGGAGT GTATCATCTA TTCTACCAGT 250
ACAACCCAAA TGGTTCAGTA TGGGGTAACA TTGTTTGGGC TCATTCCGTT 300
TCAAAAGACT TGATCAATTG GATCAATTTA GAACCTGCAA TTTACCCATC 350
AAAGCCATTT GATCAATTCG GTACCTGGTC TGGATCAGCA ACCATCCTAC 400
CTGGTAACAA GCCAGTCATC TTGTACACCG GAATCATAGA TGCCAACCAA 450
ACCCAAGTCC AAAACTACGC AATCCCAGCT AACTTATCCG ATCCATATCT 500
CCGCGAATGG ATCAAGCCAG ACAACAACCC ATTAATTATA GCCGATGAAA 550
GTATCAACAA GACCAAGTTT CGTGACCCAA CAACAGCATG GATGGGTAAA 600
GACGGGCATT GGAGAATCGT CATGGGAAGT TTGAGGAAAC ACAGCAGGGG 650
CTTAGCTATA ATGTATAGGA GCAAAGACTT TATGAAATGG GTCAAGGCTA 700
AACACCCACT TCACTCAACT AACGGCACTG GAAACTGGGA ATGCCCTGAT 750
TTTTACCCAG TTTCATCGAA AGGTACTGAT GGGTTGGATC AATACGGTGA 800
GGAACACAAG TACGTGCTGA AGAACAGTAT GGATCTTACT CGATTTGAGT 850
ATTATACACT TGGAAAATAC GATACGAAAA AAGATAGGTA CGTTCCAGAT 900
CCAGATTCTG TCGATAGTTT GAAGGGATTG AGACTCGATT ACGGTAACTT 950
CTACGCATCG AAGTCATTCT ACGATCCAAG CAAAAATCGA AGGGTTATCT 1000
GGGGTTGGTC TAATGAATCA GATATATTCC CAGAGGATGA TAATGCGAAG 1050
GGATGGGCTG GGATTCAATT GATTCCTCGT AAAGTATGGC TTGATCCAAG 1100
TGGTAAGCAG TTGGTTCAAT GGCCTGTGGA GGAACTAGAA ACCCTAAGAA 1150
CTCAAAAGGT TCAATTGAGC AACAAGAAGA TGAACAATGG GGAGAAGATT 1200
GAAGTTACAG GAATCACACC AGCACAGGCT GATGTTGAAG TGACATTCTC 1250
ATTTGCAAGT TTGGATAAGG CAGAGTCATT TGATCCTAAA TGGAATGATA 1300
TGTATGCACA AGATGTTTGT GGACTCAAGG GTGCAGATGT TCAAGGTGGG 1350
CTTGGGCCAT TTGGTCTTGC TACATTAGCT ACTGAAAACT TGGAAGAAAA 1400
CACACCGGTT TTCTTCCGAG TTTTCAAAGC ACAGCAAAAC TACAAGGTTC 1450
TCTTGTGTTC TGACGCTAAA AGGTCAACTC TTAAGTTCAA TGAAACAATG 1500
TACAAAGCTT CATTTGCTGG ATTTGTTGAT GTTGATTTGG CTGACAAGAA 1550
ATTGTCACTC AGAAGCTTGA TTGATAATTC AGTTATAGAA ACTTTTGGTG 1600
CTGGTGGAAA GACATGTATA ACATCGAGGG TTTATCCAAC ATTGGCAATT 1650
AACGACGAGG CACATTTATT CGCGTTTAAC AACGGAACGG AGCCAATCAC 1700
AATTGAGAGT TTGGATGCAT GGAGTATGGG CAAAGCTAAG ATACAATATT 1750
GA                                                   1752
```

(2)   INFORMATION FOR SEQ ID NO:12:
        (i)   SEQUENCE CHARACTERISTICS:
                (A)   LENGTH: 512
                (B)   TYPE: amino acid
                (C)   STRANDEDNESS: single
                (D)   TOPOLOGY: linear
        (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Tyr Phe Asn Gly Val Tyr His Leu Phe Tyr Gln Tyr Asn Pro
                5                   10                  15
Asn Gly Ser Val Trp Gly Asn Ile Val Trp Ala His Ser Val Ser
                20                  25                  30
Lys Asp Leu Ile Asn Trp Ile Asn Leu Glu Pro Ala Ile Tyr Pro
                35                  40                  45
Ser Lys Pro Phe Asp Gln Phe Gly Thr Trp Ser Gly Ser Ala Thr
                50                  55                  60
Ile Leu Pro Gly Asn Lys Pro Val Ile Leu Tyr Thr Gly Ile Ile
                65                  70                  75
Asp Ala Asn Gln Thr Gln Val Gln Asn Tyr Ala Ile Pro Ala Asn
                80                  85                  90
Leu Ser Asp Pro Tyr Leu Arg Glu Trp Ile Lys Pro Asp Asn Asn
                95                  100                 105
Pro Leu Ile Ile Ala Asp Glu Ser Ile Asn Lys Thr Lys Phe Arg
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                      110                     115                     120
      Asp Pro Thr Thr Ala Trp Met Gly Lys Asp Gly His Trp Arg Ile
                      125                     130                     135
      Val Met Gly Ser Leu Arg Lys His Ser Arg Gly Leu Ala Ile Met
                      140                     145                     150
      Tyr Arg Ser Lys Asp Phe Met Lys Trp Val Lys Ala Lys His Pro
                      155                     160                     165
      Leu His Ser Thr Asn Gly Thr Gly Asn Trp Glu Cys Pro Asp Phe
                      170                     175                     180
      Tyr Pro Val Ser Ser Lys Gly Thr Asp Gly Leu Asp Gln Tyr Gly
                      185                     190                     195
      Glu Glu His Lys Tyr Val Leu Lys Asn Ser Met Asp Leu Thr Arg
                      200                     205                     210
      Phe Glu Tyr Tyr Thr Leu Gly Lys Tyr Asp Thr Lys Lys Asp Arg
                      215                     220                     225
      Tyr Val Pro Asp Pro Asp Ser Val Asp Ser Leu Lys Gly Leu Arg
                      230                     235                     240
      Leu Asp Tyr Gly Asn Phe Tyr Ala Ser Lys Ser Phe Tyr Asp Pro
                      245                     250                     255
      Ser Lys Asn Arg Arg Val Ile Trp Gly Trp Ser Asn Glu Ser Asp
                      260                     265                     270
      Ile Phe Pro Glu Asp Asp Asn Ala Lys Gly Trp Ala Gly Ile Gln
                      275                     280                     285
      Leu Ile Pro Arg Lys Val Trp Leu Asp Pro Ser Gly Lys Gln Leu
                      290                     295                     300
      Val Gln Trp Pro Val Glu Glu Leu Glu Thr Leu Arg Thr Gln Lys
                      305                     310                     315
      Val Gln Leu Ser Asn Lys Lys Met Asn Asn Gly Glu Lys Ile Glu
                      320                     325                     330
      Val Thr Gly Ile Thr Pro Ala Gln Ala Asp Val Glu Val Thr Phe
                      335                     340                     345
      Ser Phe Ala Ser Leu Asp Lys Ala Glu Ser Phe Asp Pro Lys Trp
                      350                     355                     360
      Asn Asp Met Tyr Ala Gln Asp Val Cys Gly Leu Lys Gly Ala Asp
                      365                     370                     375
      Val Gln Gly Gly Leu Gly Pro Phe Gly Leu Ala Thr Leu Ala Thr
                      380                     385                     390
      Glu Asn Leu Glu Glu Asn Thr Pro Val Phe Phe Arg Val Phe Lys
                      395                     400                     405
      Ala Gln Gln Asn Tyr Lys Val Leu Leu Cys Ser Asp Ala Lys Arg
                      410                     415                     420
      Ser Thr Leu Lys Phe Asn Glu Thr Met Tyr Lys Ala Ser Phe Ala
                      425                     430                     435
      Gly Phe Val Asp Val Asp Leu Ala Asp Lys Lys Leu Ser Leu Arg
                      440                     445                     450
      Ser Leu Ile Asp Asn Ser Val Ile Glu Thr Phe Gly Ala Gly Gly
                      455                     460                     465
      Lys Thr Cys Ile Thr Ser Arg Val Tyr Pro Thr Leu Ala Ile Asn
                      470                     475                     480
      Asp Glu Ala His Leu Phe Ala Phe Asn Asn Gly Thr Glu Pro Ile
                      485                     490                     495
      Thr Ile Glu Ser Leu Asp Ala Trp Ser Met Gly Lys Ala Lys Ile
                      500                     505                     510
      Gln Tyr
```

(2)   INFORMATION FOR SEQ ID NO:13:

      (i)   SEQUENCE CHARACTERISTICS:

           (A)   LENGTH: 583

```
            (B)      TYPE:           amino acid
            (C)      STRANDEDNESS:   single
            (D)      TOPOLOGY:       linear
       (xi)     SEQUENCE DESCRIPTION:  SEQ ID NO:13:
Met Glu Leu Phe Met Lys Asn Ser Ser Leu Trp Gly Leu Lys Phe
                  5                  10                  15
Tyr Leu Phe Cys Leu Phe Ile Ile Leu Ser Asn Ile Asn Arg Ala
                 20                  25                  30
Phe Ala Ser His Asn Ile Phe Leu Asp Leu Gln Ser Ser Ser Ala
                 35                  40                  45
Ile Ser Val Lys Asn Val His Arg Thr Arg Phe His Phe Gln Pro
                 50                  55                  60
Pro Lys His Trp Ile Asn Asp Pro Asn Ala Pro Met Tyr Phe Asn
                 65                  70                  75
Gly Val Tyr His Leu Phe Tyr Gln Tyr Asn Pro Asn Gly Ser Val
                 80                  85                  90
Trp Gly Asn Ile Val Trp Ala His Ser Val Ser Lys Asp Leu Ile
                 95                 100                 105
Asn Trp Ile Asn Leu Glu Pro Ala Ile Tyr Pro Ser Lys Pro Phe
                110                 115                 120
Asp Gln Phe Gly Thr Trp Ser Gly Ser Ala Thr Ile Leu Pro Gly
                125                 130                 135
Asn Lys Pro Val Ile Leu Tyr Thr Gly Ile Ile Asp Ala Asn Gln
                140                 145                 150
Thr Gln Val Gln Asn Tyr Ala Ile Pro Ala Asn Leu Ser Asp Pro
                155                 160                 165
Tyr Leu Arg Glu Trp Ile Lys Pro Asp Asn Asn Pro Leu Ile Ile
                170                 175                 180
Ala Asp Glu Ser Ile Asn Lys Thr Lys Phe Arg Asp Pro Thr Thr
                185                 190                 195
Ala Trp Met Gly Lys Asp Gly His Trp Arg Ile Val Met Gly Ser
                200                 205                 210
Leu Arg Lys His Ser Arg Gly Leu Ala Ile Met Tyr Arg Ser Lys
                215                 220                 225
Asp Phe Met Lys Trp Val Lys Ala Lys His Pro Leu His Ser Thr
                230                 235                 240
Asn Gly Thr Gly Asn Trp Glu Cys Pro Asp Phe Tyr Pro Val Ser
                245                 250                 255
Ser Lys Gly Thr Asp Gly Leu Asp Gln Tyr Gly Glu Glu His Lys
                260                 265                 270
Tyr Val Leu Lys Asn Ser Met Asp Leu Thr Arg Phe Glu Tyr Tyr
                275                 280                 285
Thr Leu Gly Lys Tyr Asp Thr Lys Lys Asp Arg Tyr Val Pro Asp
                290                 295                 300
Pro Asp Ser Val Asp Ser Leu Lys Gly Leu Arg Leu Asp Tyr Gly
                305                 310                 315
Asn Phe Tyr Ala Ser Lys Ser Phe Tyr Asp Pro Ser Lys Asn Arg
                320                 325                 330
Arg Val Ile Trp Gly Trp Ser Asn Glu Ser Asp Ile Phe Pro Glu
                335                 340                 345
Asp Asp Asn Ala Lys Gly Trp Ala Gly Ile Gln Leu Ile Pro Arg
                350                 355                 360
Lys Val Trp Leu Asp Pro Ser Gly Lys Gln Leu Val Gln Trp Pro
                365                 370                 375
Val Glu Glu Leu Glu Thr Leu Arg Thr Gln Lys Val Gln Leu Ser
                380                 385                 390
Asn Lys Lys Met Asn Asn Gly Glu Lys Ile Glu Val Thr Gly Ile
                395                 400                 405
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Thr Pro Ala Gln Ala Asp Val Glu Val Thr Phe Ser Phe Ala Ser
            410                 415                 420
Leu Asp Lys Ala Glu Ser Phe Asp Pro Lys Trp Asn Asp Met Tyr
            425                 430                 435
Ala Gln Asp Val Cys Gly Leu Lys Gly Ala Asp Val Gln Gly Gly
            440                 445                 450
Leu Gly Pro Phe Gly Leu Ala Thr Leu Ala Thr Glu Asn Leu Glu
            455                 460                 465
Glu Asn Thr Pro Val Phe Phe Arg Val Phe Lys Ala Gln Gln Asn
            470                 475                 480
Tyr Lys Val Leu Leu Cys Ser Asp Ala Lys Arg Ser Thr Leu Lys
            485                 490                 495
Phe Asn Glu Thr Met Tyr Lys Ala Ser Phe Ala Gly Phe Val Asp
            500                 505                 510
Val Asp Leu Ala Asp Lys Lys Leu Ser Leu Arg Ser Leu Ile Asp
            515                 520                 525
Asn Ser Val Ile Glu Thr Phe Gly Ala Gly Gly Lys Thr Cys Ile
            530                 535                 540
Thr Ser Arg Val Tyr Pro Thr Leu Ala Ile Asn Asp Glu Ala His
            545                 550                 555
Leu Phe Ala Phe Asn Asn Gly Thr Glu Pro Ile Thr Ile Glu Ser
            560                 565                 570
Leu Asp Ala Trp Ser Met Gly Lys Ala Lys Ile Gln Tyr
            575                 580
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(2)   INFORMATION FOR SEQ ID NO:14:
         (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 5
                  (B)   TYPE: amino acid
                  (C)   STRANDEDNESS: single
                  (D)   TOPOLOGY: linear
         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:14:
Asn Asp Pro Asn Gly
                  5

(2)   INFORMATION FOR SEQ ID NO:15:
         (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 6
                  (B)   TYPE: amino acid
                  (C)   STRANDEDNESS: single
                  (D)   TOPOLOGY: linear
         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:15:
Trp Glu Cys Pro Asp Phe
                  5

(2)   INFORMATION FOR SEQ ID NO:16:
         (i)   SEQUENCE CHARACTERISTICS:
                  (A)   LENGTH: 500
                  (B)   TYPE: nucleic acid
                  (C)   STRANDEDNESS: double
                  (D)   TOPOLOGY: linear
         (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:16:
```
TTCTGCAAGA AGATAAAATA ATATAATTTT TTCTAGTAAT TTAAATATTA 50
TATGTGAATA TTGTAAGTTA AACATGAAGT TCACAAGGAG AGTATATGAT 100
TATATGATTA ATTAAAGATT TAGACAAAAT TAAAGGGTAT TTTTGGTACG 150
ACGTAAAAAT AACTTTTTAG AAAATATTTT TGCGAGTATA TTATTTATTA 200
ATGTTTTATA CTAATATAGA AGTCGTTATT TTTAGGGAAA AAAAGTTCTT 250
TTCAAAATAT GAAATAAATT TCTAGCCTAG GGACGAAAGT CTTTTTTTTT 300
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TTTATAACTA TAGTAAACGT AAAATCACGT AATTAAAACA TTTATAATAA 350
TAAAAGATAA AAGATCTATA TTGGTTTTAC CAATTAGTAC ATATTAGGTT 400
TTAGTCACGT TAATATGTTT ACTTTTTTGT TCTAATATTA GTAATTATCT 450
ATTAATCTTG TAATAGCTAA TTTTTTTATT TTTTTTTTGT AATTGATTAA 500
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WHAT IS CLAIMED IS:

1.   An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino acid sequence serving for secretion into an apoplast.

2.   The isolated nucleic acid of claim 1, wherein said polypeptide is at least 80 % homologous to SEQ ID NOs:6 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

3.   The isolated nucleic acid of claim 1, wherein said polynucleotide is hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5×10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

4.   The isolated nucleic acid of claim 1, wherein said polynucleotide is at least 80 % identical with SEQ ID NOs:7 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

5.   The isolated nucleic acid of claim 1, wherein said polypeptide is as set forth in SEQ ID NOs:6 or 13 or portions thereof.

6.   The isolated nucleic acid of claim 1, wherein said polynucleotide is as set forth in SEQ ID NOs:7 or 11 or portions thereof.

7.   An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity, said polypeptide is at least 80 % homologous to SEQ ID NOs:6, 12 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith

and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

8.   The isolated nucleic acid of claim 7, wherein said polynucleotide is hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

9.   The isolated nucleic acid of claim 7, wherein said polynucleotide is at least 80 % identical with SEQ ID NOs:7, 9 or 11 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

10.   The isolated nucleic acid of claim 7, wherein said polypeptide is as set forth in SEQ ID NOs:6, 12 or 13 or portions thereof.

11. The isolated nucleic acid of claim 7, wherein said polynucleotide is as set forth in SEQ ID NOs:7, 9 or 11 or portions thereof.

12. A nucleic acid construct comprising the isolated nucleic acid of claim 1.

13. The nucleic acid construct of claim 12, further comprising a promoter for regulating expression of the isolated nucleic acid in an orientation selected from the group consisting of sense and antisense orientation.

14. The nucleic acid construct of claim 12, further comprising a positive and a negative selection markers for selecting for homologous recombination events.

15. A plant cell, tissue or a whole plant comprising the nucleic acid construct of claim 12.

16.   A nucleic acid construct comprising the isolated nucleic acid of claim 7.

17.   The nucleic acid construct of claim 16, further comprising a promoter for regulating expression of the isolated nucleic acid in an orientation selected from the group consisting of sense and antisense orientation.

18.   The nucleic acid construct of claim 16, further comprising a positive and a negative selection markers for selecting for homologous recombination events.

19.   A plant cell, tissue or a whole plant comprising the nucleic acid construct of claim 16.

20.   An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 %

SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash

solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

21.   An isolated nucleic acid comprising a genomic,

complementary or composite polynucleotide sequence at least 80 %

identical with SEQ ID NOs:1, 5, 7, 8, 9 or 11 as determined using the

BestFit software of the Wisconsin sequence analysis package, utilizing

the Smith and Waterman algorithm, where gap weight equals 50, length

weight equals 3, average match equals 10 and average mismatch equals -

9.

22.   An isolated nucleic acid comprising a polynucleotide

sequence as set forth in SEQ ID NOs:1, 5, 7, 8, 9 or 11.

23.   An isolated nucleic acid comprising a polynucleotide

sequence encoding a polypeptide as set forth in SEQ ID NOs:6, 12 or 13.

24.   A recombinant protein comprising a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino acid sequence serving for secretion into an apoplast.

25.   The recombinant protein of claim 24, wherein said polypeptide is at least 80 % homologous to SEQ ID NOs:6 or 13, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

26.   The recombinant protein of claim 24, wherein said polypeptide includes at least a portion of SEQ ID NOs:6 or 13.

27.   The recombinant protein of claim 24, wherein the protein is encoded by a polynucleotide hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 or a portion thereof under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

28. The recombinant protein of claim 24, wherein the protein is encoded by a polynucleotide at least 80 % identical with SEQ ID NOs:7 or 11 or portions thereof as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

29. A recombinant protein comprising a polypeptide as set forth in SEQ ID NOs:6, 12 or 13.

30. A recombinant protein comprising a polypeptide at least 80 % homologous to SEQ ID NOs:6, 12 or 13 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

31. A method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing in the plant tissue a polypeptide having invertase activity, wherein said polypeptide is

at least 80 % homologous to SEQ ID NOs:6, 12 or 13 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2.

32.   A method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing a polypeptide having invertase activity, wherein said polypeptide is encoded by a polynucleotide hybridizable with SEQ ID NOs:1, 5, 7, 8, 9 or 11 or a portion thereof under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.1 x SSC and 0.1 % SDS and final wash at 60 °C.

33.   A method of increasing a level of a monosaccharide in a plant tissue, the method comprising the step of expressing a polypeptide having invertase activity, wherein said polypeptide is encoded by a polynucleotide at least 80 % identical with SEQ ID NOs:7, 9 or 11 as determined using the BestFit software of the Wisconsin sequence

analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

34. An isolated regulatory element comprising a polynucleotide at least 50 % identical with SEQ ID NO:4 as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals - 9.

35. An isolated regulatory element comprising a polynucleotide hybridizable with SEQ ID NO:4 under hybridization conditions of hybridization solution containing 10 % dextrane sulfate, 1 M NaCl, 1 % SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 1 x SSC and 0.1 % SDS and final wash at 50 °C.

36. An expression vector including the isolated regulatory element of claim 34.

37.   A method of increasing a level of a monosaccharide in a tissue of a solanaceae plant, the method comprising the step of integrating into a genome of the solanaceae plant a polynucleotide including a nucleic acid sequence as set forth in SEQ ID NO:4, wherein said polynucleotide is integrated into a specific site of chromosome 9 of the solanaceae plant via homologous recombination.

38.   A method for determining whether fruits to be produced from solanaceae seeds or solanaceae seedling will contain an amount of monosaccharides above a predetermined threshold, the method comprising the step of detecting the presence or absence of a nucleic acid sequence as set forth in SEQ ID NO:4 in genomic DNA derived from the solanaceae seeds or solanaceae seedling.

ABSTRACT OF THE DISCLOSURE

An isolated nucleic acid comprising a genomic, complementary or composite polynucleotide sequence encoding a polypeptide having an invertase activity in an apoplastic environment and an N terminal amino acid sequence serving for secretion into an apoplast.

Combined Declaration For Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled   <u>POLYNUCLEOTIDES ENCODING POLYPEPTIDES HAVING INVERTASE ACTIVITY AND USE OF SAME</u>,   the specification of which

(check one)   ☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____ and was amended on _____. I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
<u> NONE </u>	<u> </u>	<u> </u>	☐	☐
(number)	(Country)	(Day, Month, Year Filed)	Yes	No
<u> </u>	<u> </u>	<u> </u>	☐	☐
(number)	(Country)	(Day, Month, Year Filed)	Yes	No
<u> </u>	<u> </u>	<u> </u>	☐	☐
(number)	(Country)	(Day, Month, Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u> NONE </u>   <u>   </u>   —
(Application Serial No.)   (Filing Date)   Status
                                        (patented, pending, abandoned)

<u>   </u>   <u>   </u>   —
(Application Serial No.)   (Filing Date)   Status
                                        (patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Mark M. Friedman   Registration No. 33,883

Address all Correspondence to:

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ARLINGTON, VIRGINIA 22202	

Attorney Docket: <u>325/79</u>
page 2 of 2

Continuation of Combined Declaration For Patent Application and Power of Attorney

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application of any patent issued thereon.

*FULL NAME OF SOLE OR FIRST INVENTOR DANI ZAMIR	INVENTOR'S SIGNATURE *(signed)*	DATE 29/12/99
RESIDENCE 10 DERECH ERETZ, GEDERA, ISRAEL	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS 10 DERECH ERETZ, GEDERA, ISRAEL		

*FULL NAME OF SECOND INVENTOR TZILI PLEBAN	INVENTOR'S SIGNATURE *Tzili Pleban*	DATE 29.12.99
RESIDENCE 123 STERN, KIRIAT ONO, ISRAEL	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS 123 STERN, KIRIAT ONO, ISRAEL		

*FULL NAME OF THIRD INVENTOR EYAL FRIDMAN	INVENTOR'S SIGNATURE *Eyal Fridman*	DATE 28.12.99
RESIDENCE 32 SIRENY, REHOVOT, ISRAEL	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS 32 SIRENY, REHOVOT, ISRAEL		

*FULL NAME OF FOURTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF FIFTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF SIXTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

*FULL NAME OF SEVENTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP ISRAELI	
POST OFFICE ADDRESS		

SMALL BUSINESS CONCERN - NEW APPLICATION

Attorney Docket No.: 325/78

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In RE Application of: DANI ZAMIR ET AL.

Filed Concurrently Herewith

For: POLYNUCLEOTIDES ENCODING POLYPEPTIDES HAVING INVERTASE ACTIVITY AND USE OF SAME

VERIFIED STATEMENT UNDER 37 CFR 1.27
CLAIMING STATUS AS A SMALL ENTITY

To The Commissioner of Patents and Trademarks:

I hereby declare that:

I am the owner of, or an official empowered to act on behalf of, the small business concern identified below:

Name of Concern: YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM

Address   : 46 JABOTINSKI, 91042 JERUSALEM, ISRAEL

The small business concern identified above, together with its affiliates, employs fewer than 500 persons and qualifies as a small business concern as defined in 37 CFR 1.9(d) for purposes of paying reduced fees under 35 USC § 41(a) and § 41(b) to the Patent and Trademark Office with regard to the above-entitled invention described in the specification filed herewith.

Rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above entitled invention.

If the rights held by the small business concern are not exclusive, each other party having rights to the invention is listed below, and no rights to the invention are held by any party who could not qualify as a small entity under 37 CFR 1.9(f), namely any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Full Name (Party 1)   : NONE

Address   : _____

Status   : ☐ Individual   ☐ Small Business   ☐ Nonprofit
                                            Concern   Organization

Full Name (Party 2)   : _____

Address   : _____

Status   : ☐ Individual   ☐ Small Business   ☐ Nonprofit
                                            Concern   Organization

I acknowledge the duty under 37 CFR 1.28(b) to file, in this application, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the issue fee due after the date on which status as a small entity is no longer appropriate.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

NURIT INBAR   N. Inbar   DECEMBER 28, 1999
Name of Person Signing   Signature   Date

Capacity of Person Signing: ADMINISTRATIVE MANAGER

Address of Person Signing : 46 JABOTINSKI , JERUSALEM 91042

YISSUM
RESEARCH DEVELOPMENT COMPANY
OF THE
HEBREW UNIVERSITY OF JERUSALEM
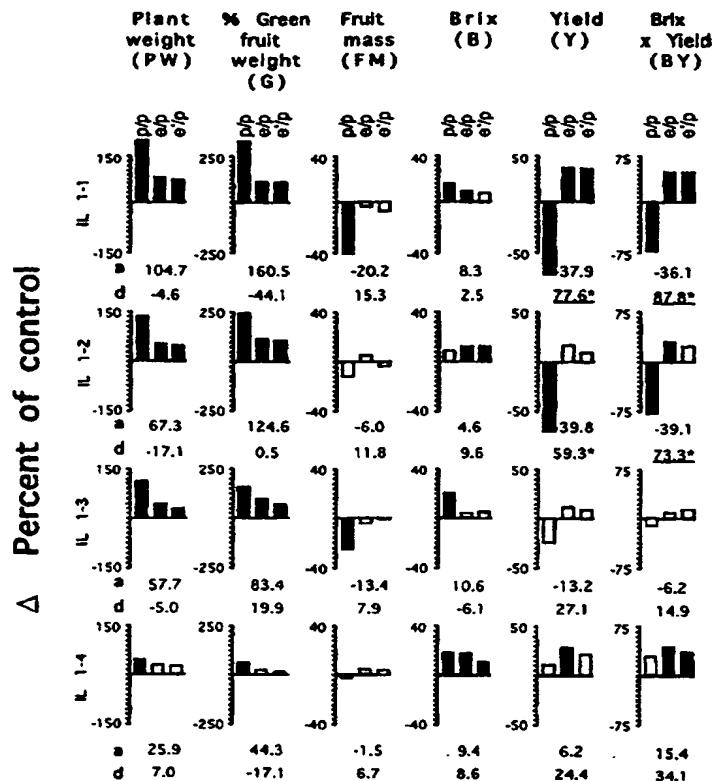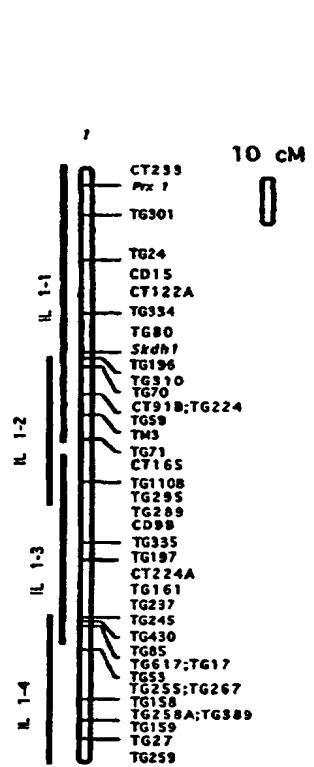
Fig 1a
(prior art)
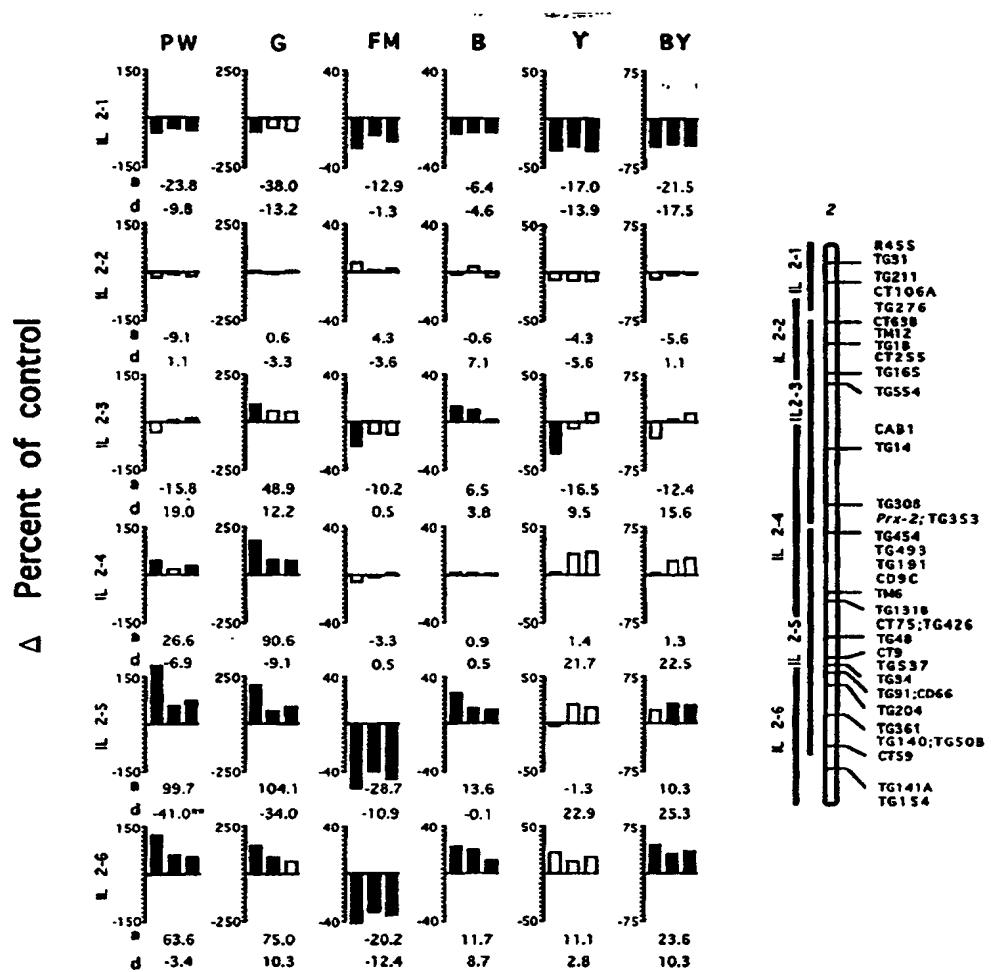
Fig 1b
(prior art)
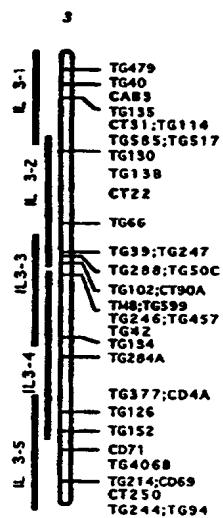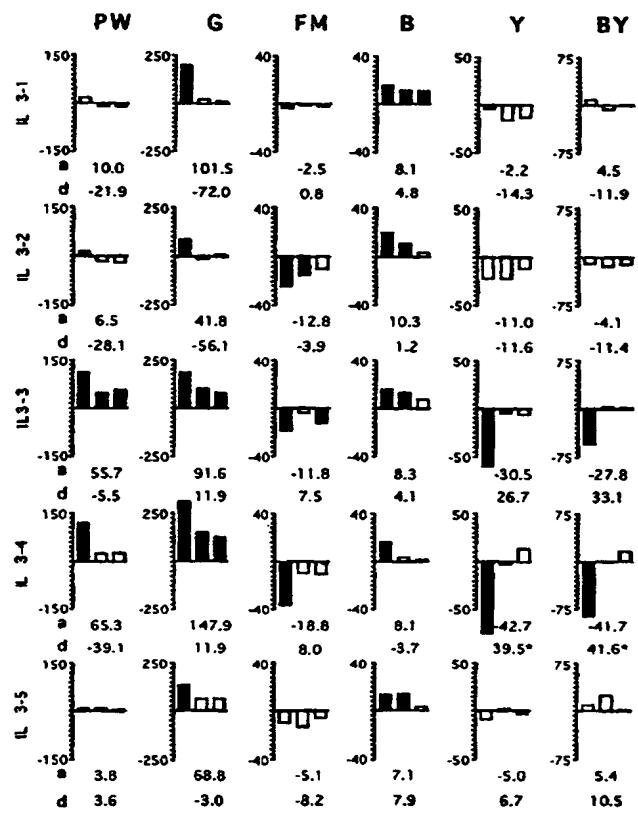
Fig 1c
(prior art)

Fig 1d
(prior art)

Δ Percent of control
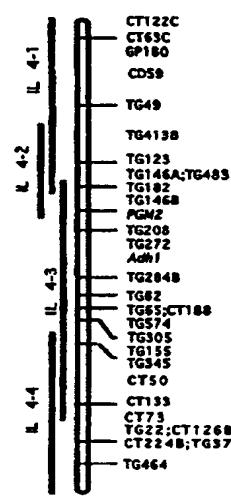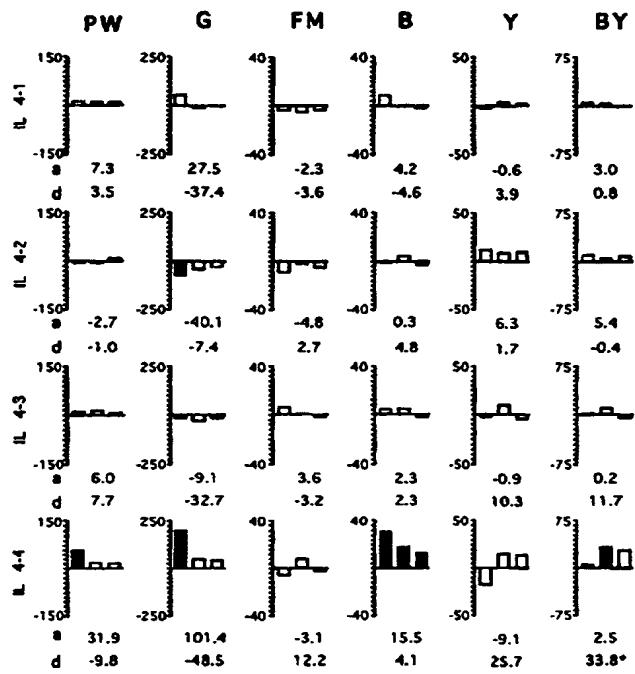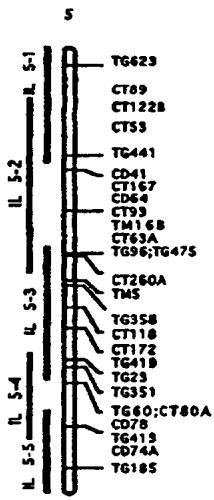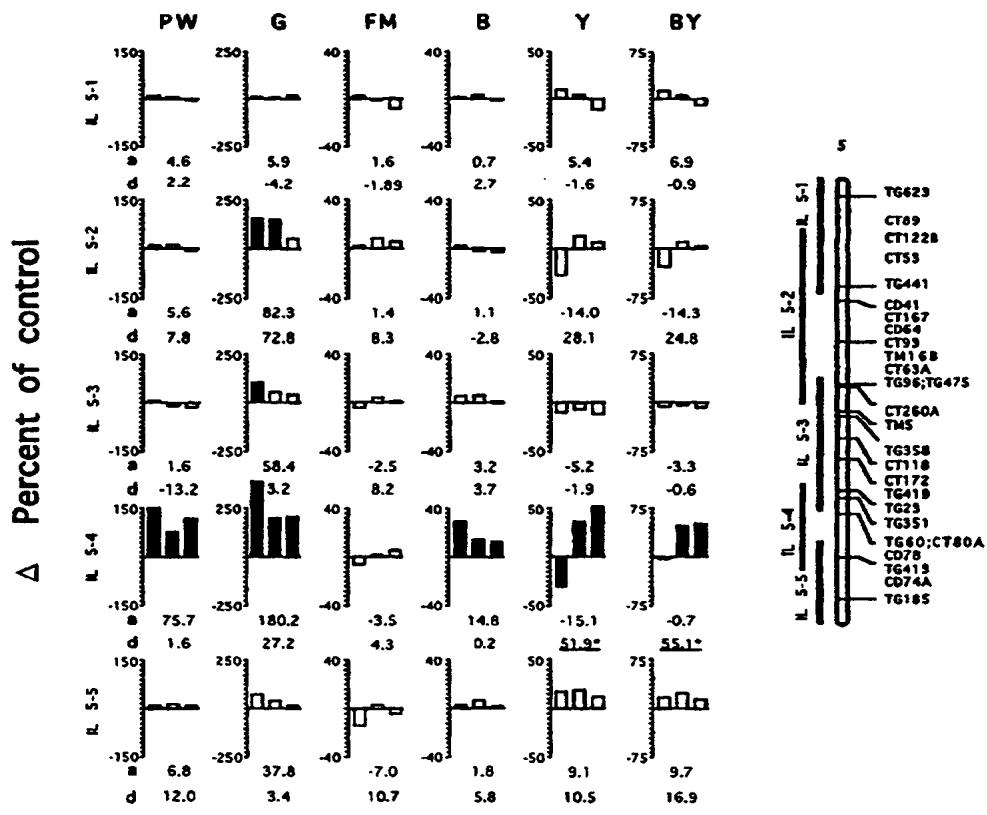
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(prior art)
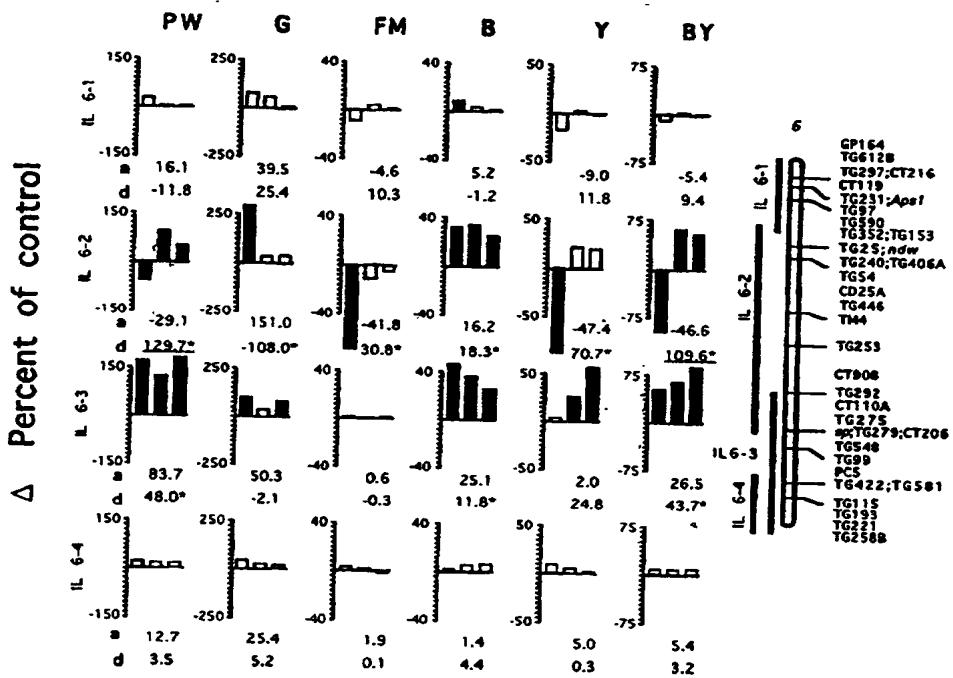
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(prior art)
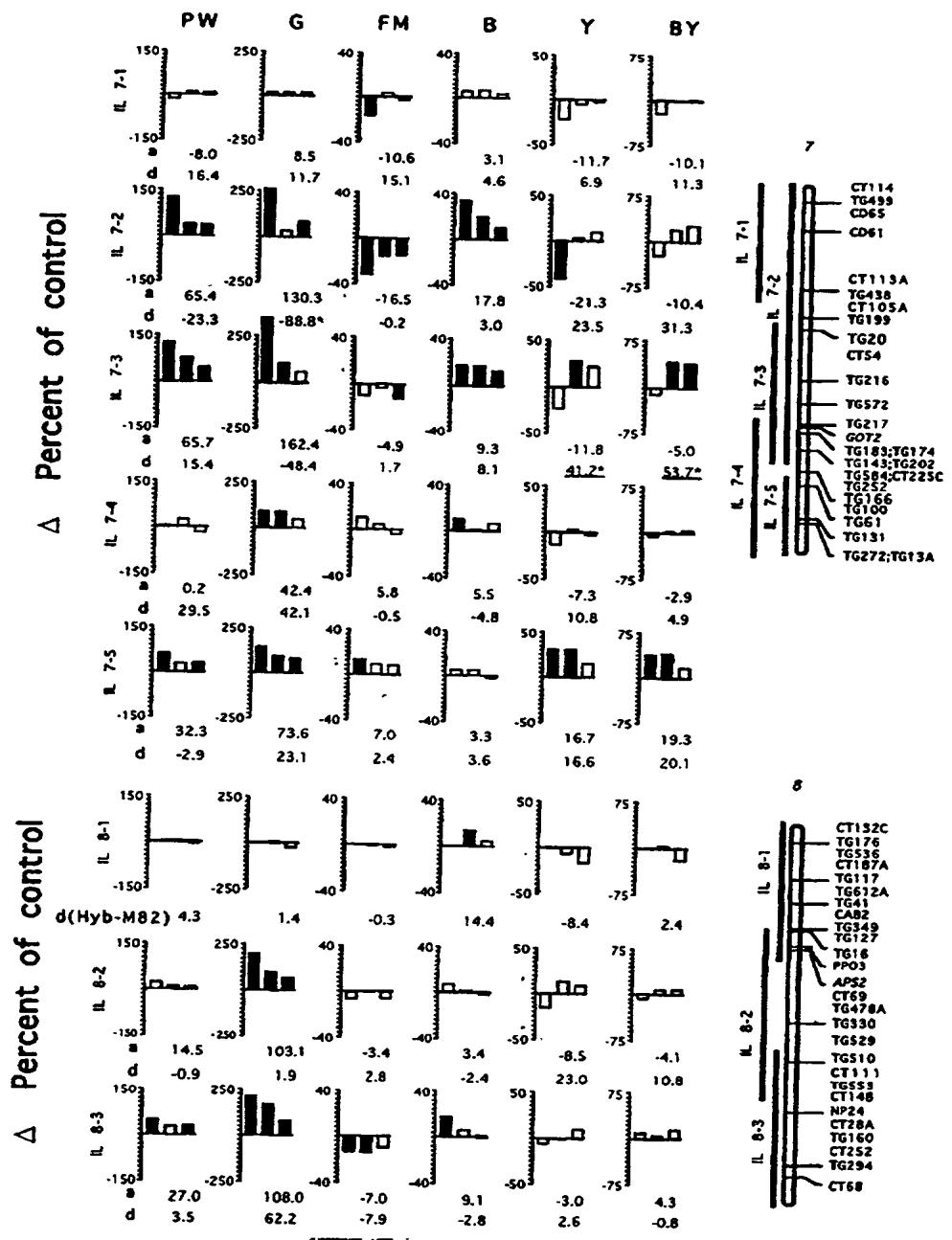
Fig 1g
(prior art)

Fig 1h

(prior art)

Δ Percent of control

	PW	G	FM	B	Y	BY
IL 11-1						
a	31.2	86.5	-13.2	6.5	-2.4	0.6
d	-10.9	25.4	0.6	0.3	15.9	18.1
IL 11-2						
a	36.9	100.5	-8.5	13.3	3.0	16.7
d	-10.1	13.8	-2.7	6.6	7.9	13.0
IL 11-3						
a	9.5	68.0	-8.4	7.3	-6.3	-1.4
d	7.9	40.0	-1.7	-0.1	15.3	17.3
IL 11-4						
a	2.7	19.4	-8.2	11.4	-4.7	4.4
d	-8.2	35.8	4.4	-2.8	-10.4	-3.0

Δ Percent of control

	PW	G	FM	B	Y	BY
IL12-1						
a	-3.5	-2.7	12.5	5.7	-4.8	-0.7
d	23.4	29.2	2.6	9.2	13.4	22.9
IL12-2						
a	5.0	10.4	-4.0	9.3	-0.4	7.5
d	3.3	-3.3	-3.0	6.3	17.5	25.8
IL12-3						
a	5.0	49.0	-0.9	9.0	6.4	15.0
d	17.5	32.3	0.9	4.6	17.6	23.1
IL12-4						
a	-15.9	-6.5	4.9	2.9	-10.7	-9.1
d	-7.6	-1.2	-4.4	0.2	-6.8	-5.3

Chromosome 11 markers (top to bottom): CT110B, TG497, CT113B, TG651, CT168, TG194, TG523J, CP58, CT182, TG147, TG46, TG562, CT107C, TG36, TG30, TG105A, TG26, CT120A, TG393, TG104 (IL 11-1, IL 11-2, IL 11-3, IL 11-4)

Chromosome 12 markers (top to bottom): TG180, Aco1, TG68, TG263A, CT19, CT120B, CT79, TG263B, CT211A, TG28B, Pgi1, CD4B, TG50A, TG111, TG565, 6Pgdh2, Est4;TM-16C, TG28A, TG296, CT80B, CT276, TG473;TG602, CD2 (IL12-1, IL12-2, IL12-3, IL12-4)

Fig 1i
(prior art)

ILH11-1
PWFM B Y BY
10 -15 6 -7 -2

ILH10-1
PWFM B Y BY
1 -17 13 -9 4

ILH9-2-5
PWFM B Y BY
14 -8 22 4 29

ILH7-5
PWFM B Y BY
35 9 6 15 24

ILH5-4
PWFM B Y BY
44 3 12 18 31

ILH2-6-1
PWFM B Y BY
47 -37 17 -3 15

ILH2-1
PWFM B Y BY
-30 -7 -12 -22 -31

ILH1-4
PWFM B Y BY
15 3 14 7 23

Fig 2
(prior art)

No. of introgression pairs

No. of traits with significant epistasis

expected
observed

Fig 3 (prior art)

Fig 4
(prior art)

Fig 6 (prior art)

| | IL9-2-5 |
| | ILH9-2-5 |

	PW			FM			Brix		
	1995	1996	1997	1995	1996	1997	1995	1996	1997
d		10.0		-4.0	1.2	-10.0*		9.0*	
a		14.0		-4.0	2.3	3.2		15.0	
control (M82)	1.8	1.8	1.2	56.1	51.3	62.9	4.5	4.9	3.6
std	0.5	0.4	0.3	5.9	4.4	5.4	0.4	0.4	0.3

Fig 7

North(telomer)

South(centromere)

1.

TG 225   CT 283   sp9   GP 263   p14.1

Chr. 9

0.1   0.24   0.33   0.12

IL 9-2-5
IL 9-2-6
IL 9-2-7

Fig 8a

2.   Brix   vegetation   % green   FM

Δ% from control (M82)

ILH 9-2-5
ILH 9-2-6
ILH 9-2-7

Fig 8b

Brix

Fig 9

Figure 10

Brix   **Glasshouse**

Fig 11

a % Soluble
solids
concentration
(Brix)

Open-field
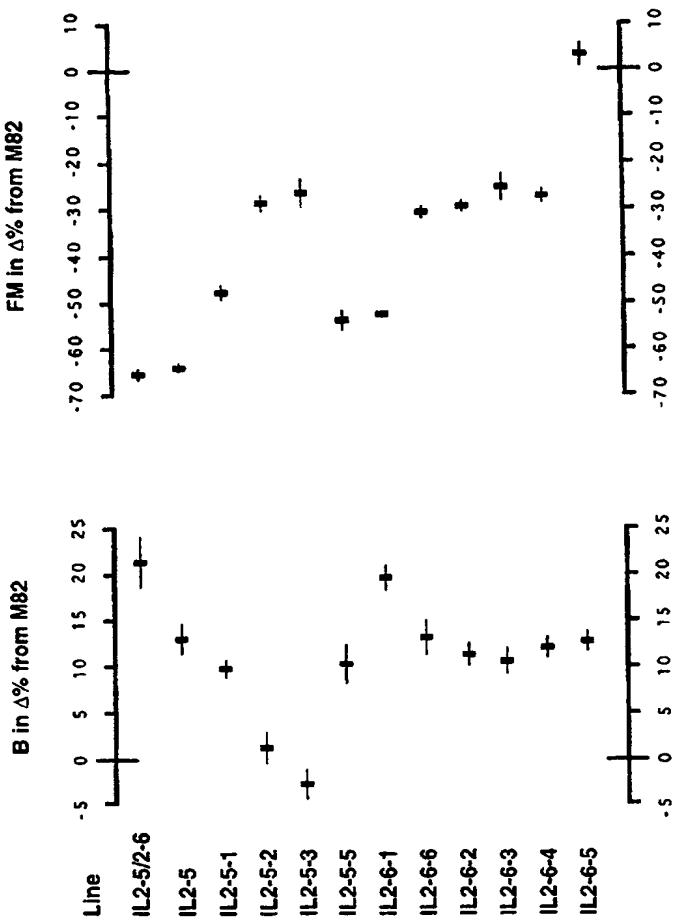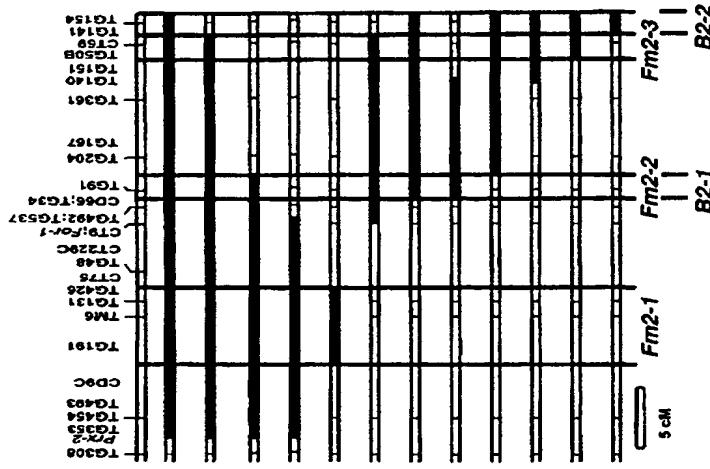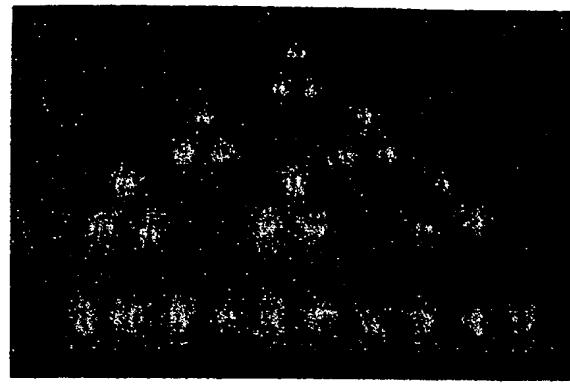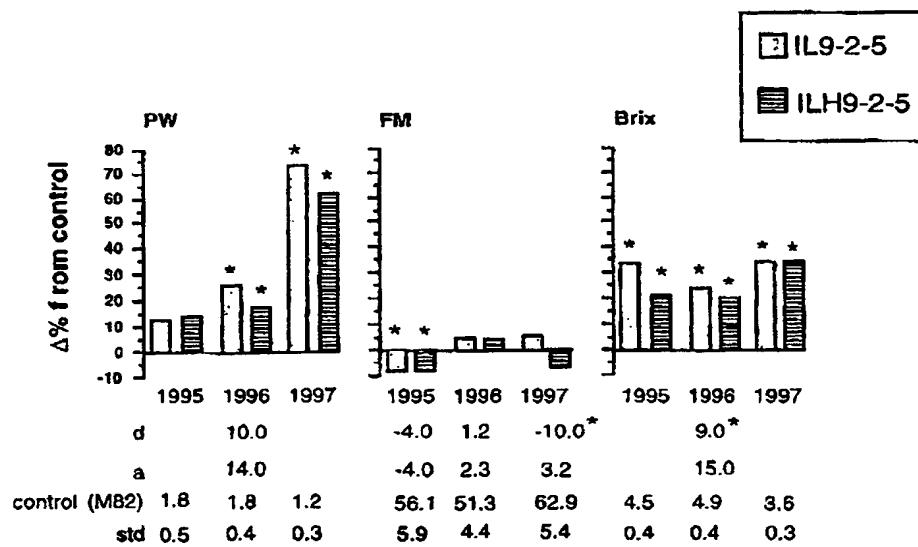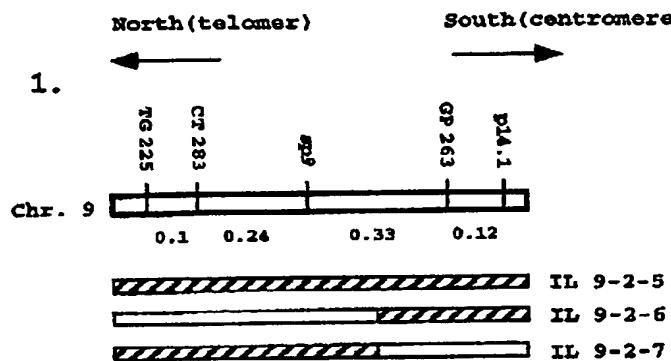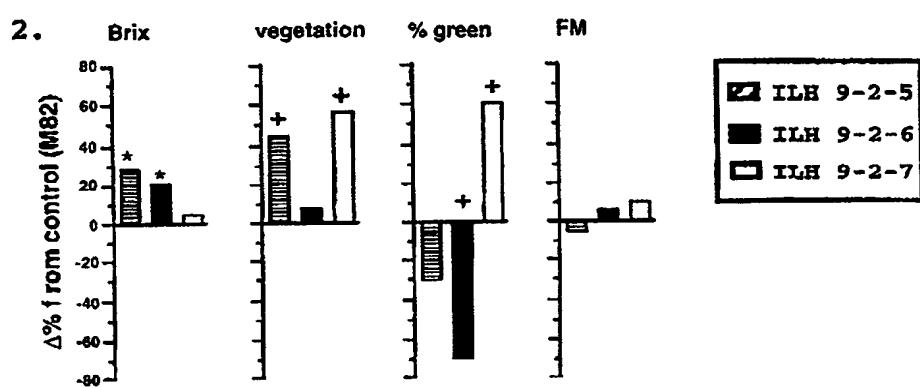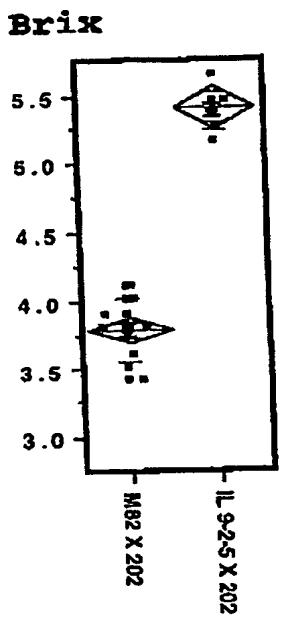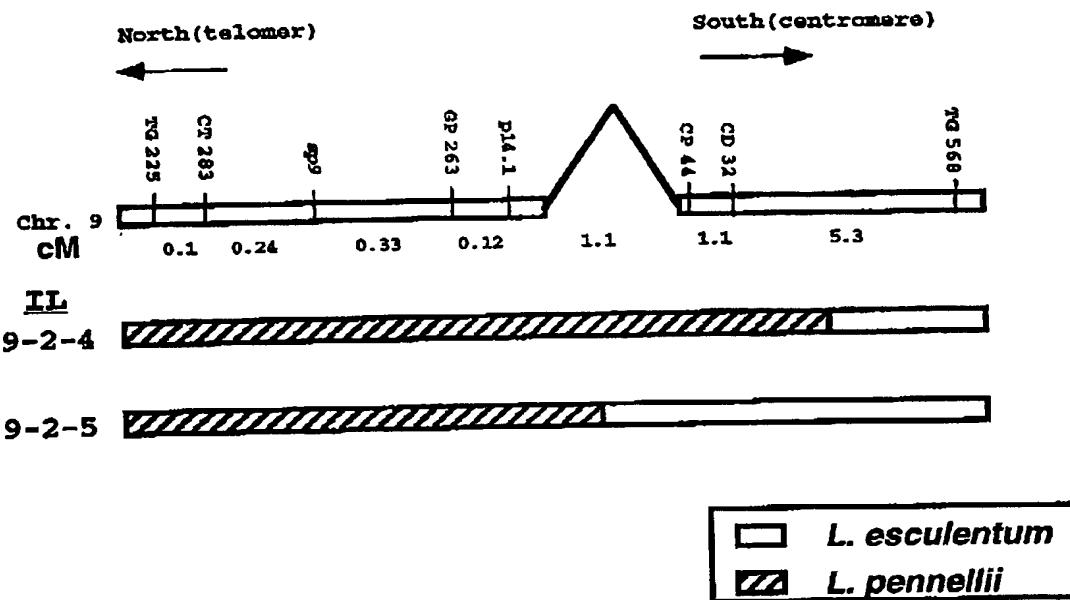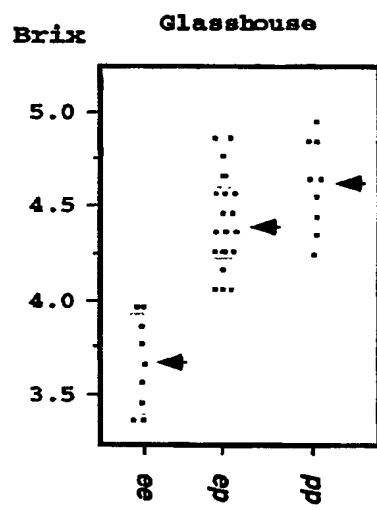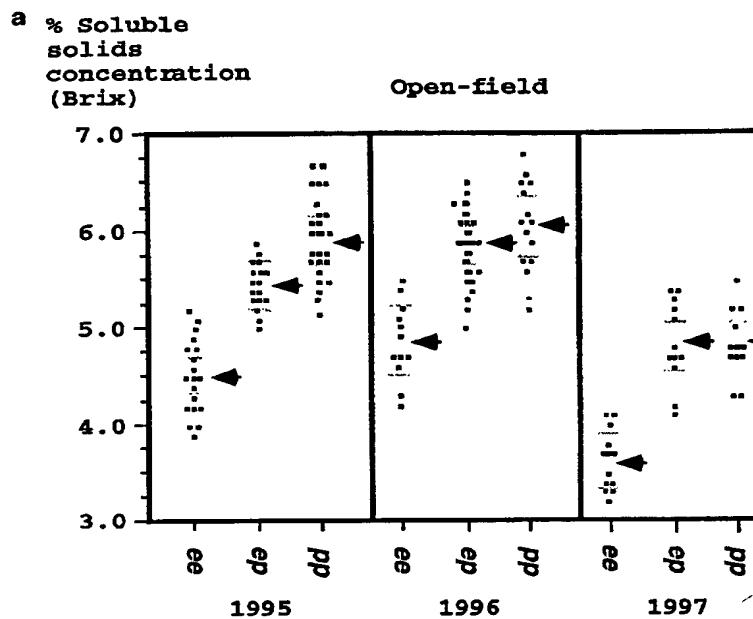
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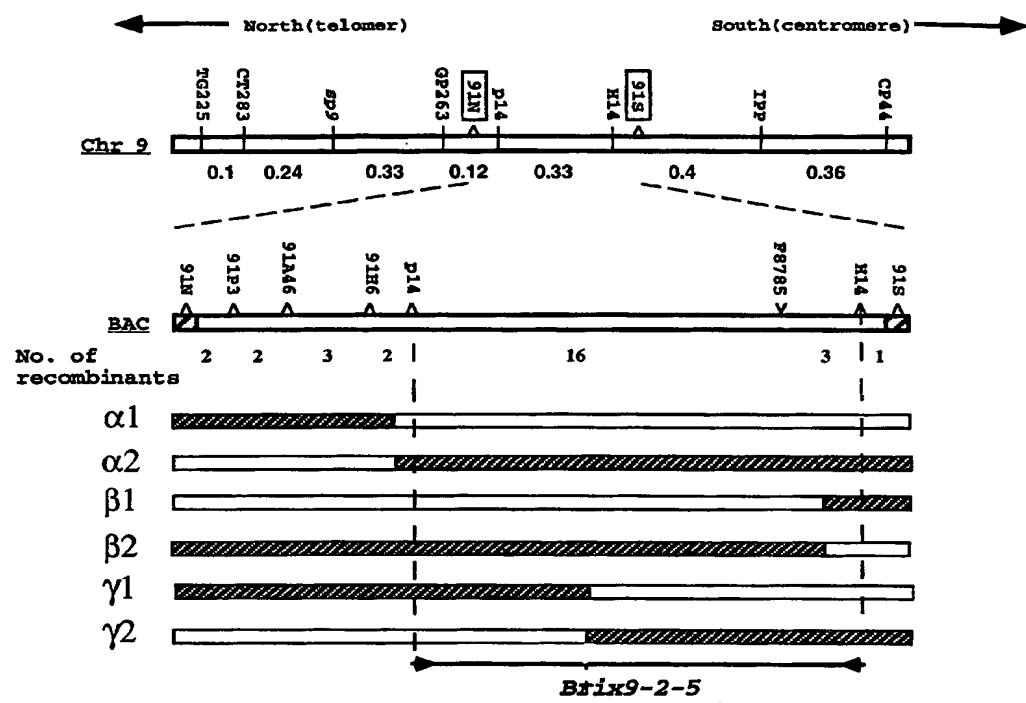
Fig 13

Fig 14a

Brix 9-2-5

Fig 14b
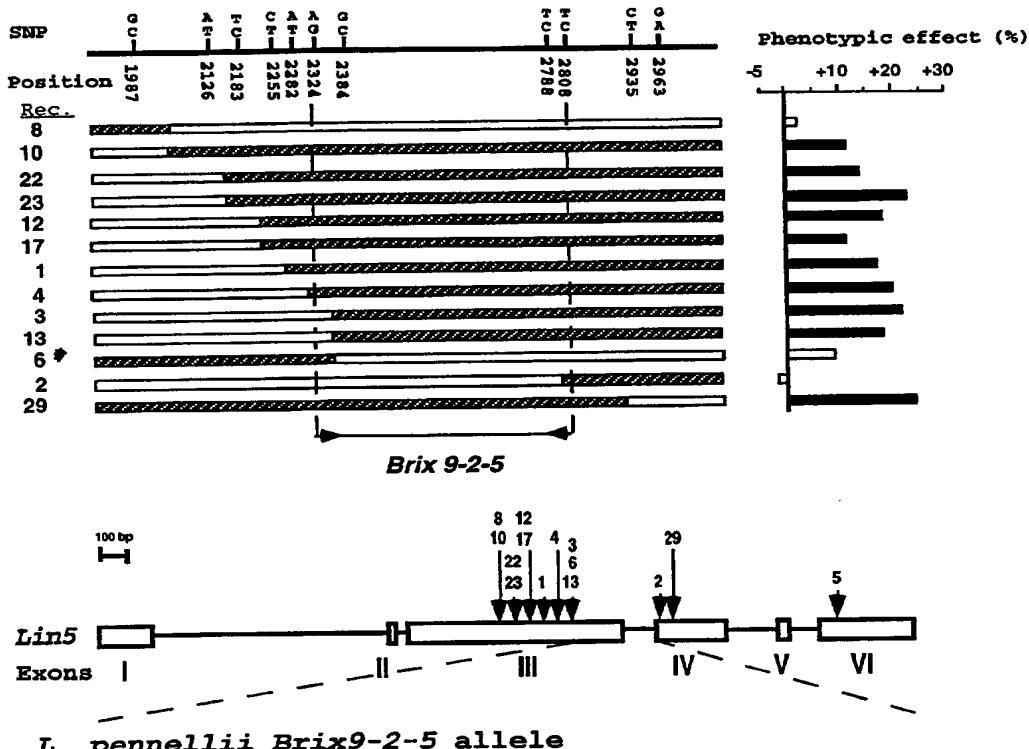
L. pennellii Brix9-2-5 allele

```
2301  GACTATGGTA ATTTCTATGC ATCAAAGACA TTCTATGATC CTAGCAGAAA
2351  TCGAAGGGTT ATTTGGGGTT GGTCAAATGA ATCGGATGTA TTACCTGACG
2401  ATGATATTAA GAAAGGATGG GCTGGAATTC AAGGTATTCC GCGACAAGTA
2451  TGGCTAGACC TTAGTGGTAA ACAATTAGTT CAATGGCCTA TTGAAGAATT
2501  AGAAACCCTA AGGAAGCAAA AGGTCCAATT GAACAACAAG AAGTTGAGCA

2551  AGGGAGAAAT GTTTGAAGTT AAAGGAATCT CAGCATCACA GGTTTCAACT

2601  TTTACTTATT AACCCATAGT CTTTTAAATA TCATTAATCT AGTTCTTATG

2651  TATACAATGT ATAATCAATG TATAACTATT ATATCAATTG CACATGATCG

2701  ATCGATATAT ATATAGTAGA TTGATTATAC ATTTGTTATA TATATCTATT

2751  ATATCAATTG CACTGTCTCA TCTTGCATTT CTTTGATTGT AGGCTGATGT

2801  TGAAGTGTTA TTCTCATTTT CAAGTTTAAA CAAGGCCGAA CAATTTGATC
```

Lin5

Exons   I   II   III   IV   V   VI   I   II

Lin7

III   IV   V   VI

Fig 15